RESEARCH PAPER

Impact of Oocyte Quality on Blastocyst Formation and Aneuploidy Detection Using Spent Media-Based NICS in prime cohort IVF Patients

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ABSTRACT

Background: Maternal age also contributes to poor quality of oocytes and increased rates of embryonic aneuploidy, which complicates assisted reproductive techniques.

Methods: This study examines the impact of oocyte morphological appearance on determining the embryonic developmental stage (blastulation) and the chromosomal status of the embryo through non-invasive chromosomal screening (NICS) based on spent culture media.

Results: Among the 460 women aged 35-45 years undergoing intracytoplasmic sperm injection (ICSI) and in vitro fertilization (IVF), 330 fulfil the inclusion criteria. A total of 3,630 MII oocytes were morphologically scored and retrieved with 37.2% being considered high quality, 33.3% morphologically moderate quality, and 29.5% being poorly graded. Out of the 3,120 fertilized embryos, 1,925 were able to survive to the blastocyst stage, which is a 61.7% developmental rate and from these embryos, 1,160 were graded as blastocysts with quality ≥3AA and ≥3BB, which is considered high to moderate quality (60.3%). Culture media from 1,890 of these blastocysts were collected and cell-free DNA was retrieved in 96% of the samples. 1,780 of these were successfully analysed with Next-Generation Sequencing (NGS) and were classified as 47.2% euploid, 37.6% aneuploid, 10.7% mosaic, and 4.5% without chromosomal parameters to call the embryo with compiled chromosomal data.

Conclusion: Oocyte quality was positively associated with the clinical pregnancy outcome ($p < 0.001$), whereas there was an association of increased risk of miscarriage ($p = 0.027$) due to aneuploidy and mosaicism. Chromosomal quality (via NICS), and blastocyst proficiency, were all highly impacted by oocyte morphology in older patients undergoing IVF.

Keywords: Oocyte morphology; Blastocyst quality; Non-invasive chromosomal screening; advanced maternal age; IVF; Aneuploidy

How to cite this article: Pendyala N, Adusumilli R, Rao PV. Impact of Oocyte Quality on Blastocyst Formation and Aneuploidy Detection Using Spent Media-Based NICS in Prime Cohort IVF Patients. Int J Drug Deliv Technol. 2026;16(17s): 649-658. DOI: 10.25258/ijddt.16.17s.75

1. INTRODUCTION

Assisted reproductive technology (ART), particularly in vitro fertilization (IVF), is the most common form of treatment for the infertility of women over 35 years of age (advanced maternal age). Progress, however, is not equal across the board, and the success of ART as measured by clinical pregnancies and live births is highly age-dependent (Ubaldi et al., 2019). This is in part due to the decline in the quality of oocytes, the maternal age factor further increasing the chances of chromosomal abnormalities occurring in embryos (Navot et al., 1991; Tesarik & Mendoza, 2003). In clinical practice, the process of unpacking oocytes from the microscope to morphologically grade them is not as fancy, but quite meaningful; they are simple, weak, and rather poor predictors. For example, oocytes are not likely to give rise to a viable blastocyst. Likewise, poorly graded oocytes are likely to undergo stunted cleavage and/or fail to form a blastocyst (Balaban & Urman, 2006; Rienzi et al., 2011).In addition to the formation of blastocysts, the genetic make-up of embryos must also be taken into account when determining the likelihood of successful implantation and continuous gestation. With maternal aging, both aneuploidy and chromosomal imbalances increase, in addition to being a key cause for both implantation failure and miscarriage, and adverse

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pregnancy outcomes (Franasiak et al., 2014). For some time, Preimplantation genetic testing for aneuploidy (PGT-A) by means of a trophectoderm biopsy has been the method of embryo screening; however, that method is both invasive and has the potential to compromise the embryo's structural integrity (Scott et al., 2013). In an attempt to obtain a better means of embryo screening, non-invasive chromosomal screening (NICS) has been a welcome replacement by using cell-free DNA (cfDNA) obtained through the culture media. With this method of ploidy assessment, embryos remain unbiopsied; therefore, embryo structures retain all of the original components, and the risks of that type of procedure are entirely obviated (Xu et al., 2016; Rubio et al., 2019).It has been researched recently that in spent media, cfDNA comes from the trophectoderm and the inner cell mass, and is of an amount that can reflect the physiological status and quality of the embryo (Vera-Rodriguez et al., 2018). However, there is various maternal and embryonic factors that could affect cfDNA release. This includes factors such as oocyte competence, dynamics of the apoptotic cell activity, and the expansion of the blastocyst. Although positive initial reports indicated that NICS could reliably tell the difference in the euploid and aneuploid embryos, it's concordance may not have an easy variance with invasive PGT-A, depending on the embryological context (Minasi et al., 2021; Chen et al., 2022;. Especially due to the reason of the initial morphological quality of the oocytes being largely unknown in how it could potentially affect the downstream blastocyst, its development, and the initial down development of the efficiency and accuracy of cfDNA-based non-invasive screening in older patients. Because of the clinical importance of embryo selection in older-age IVF cycle patients, the relationship between oocyte quality and embryo development, as well as the genetic profiling, is worth exploring. Therefore, the objective of this study was to evaluate the influence of the oocyte morphological grade on the formation rates of blastocysts and their chromosomal status, as defined by NICS through the used spent culture media. This study is also aimed at the comparison of the diagnostic performance of NICS and PGT-A from the trophectoderm biopsy taken from this specific groups of embryos. The study places emphasis on women within the 35 to 45 years range to understand the role of oocyte competence on the non-invasive detection of aneuploidy, while further enhancing the methods to choose embryos during the process of age-related infertility.

2. METHODOLOGY

2.1. Study design

The study took place at a tertiary in vitro fertilization centre (IVF) over a period of 18-24 months, after getting approval from the Institutional Ethical Committee, as a prospective, observational cohort study. Participants were ethically given the opportunity to provide informed consent before enrolment, as described in the Declaration of Helsinki. Oocytes were evaluate Morphological Quality to determine the Blastocyst Formation Rate and Quality as well as to determine the Clinical Utility and Diagnostic Validity of Non-invasive Chromosomal Screening (NICS) using cell-free DNA (cfDNA) obtained from the culture media of spent blastocysts. Due to the fact that NICS is non-invasive, it serves as a viable alternative to conventional embryo biopsy (Xu et al., 2016).

2.2. Study population

Potential participants in this study were women between the ages of 35 and 45 who enrolled in the study for undergoing in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI), had 3 or more mature MII (Metaphase II) oocytes that had been fertilized via ICSI and who agreed to culture media collection for non-invasive prenatal testing (without a trophectoderm biopsy). In this study, embryos were cultured to the blastocyst stage (5/6 Days) in individual containers. Exclusion criteria were the use of donor gametes, poor responders (i.e., those with less than 3 MII oocytes), and those who had shared culture system(s), were media contaminated, had failed to form a blastocyst, or had known parent(s) with genetic disorders. These criteria were used in an effort to have the same conditions for the study participants in order to have as few factors as possible that would impact the analysis of the cell-free DNA (cfDNA) analysis (Minasi et al., 2021).

2.3. Ovarian stimulation and oocyte quality assessment

Controlled ovarian stimulation (COS) was performed using tailored (individualized) antagonist or long agonist protocols following ovarian reserve evaluation (anti-mullerian hormone (AMH) and antral follicle count (AFC)). Selective follicular growth was tracked using serial transvaginal ultrasounds and serum estradiol concentrations. Either human chorionic gonadotropin (hCG) or a gonadotropin-releasing hormone (GnRH) agonist was administered to trigger final maturation of oocytes (oocyte maturation) before oocyte (egg) retrieval 36 hours later, when leading (dominant) follicles were at or above 18 mm. Cumulus-oocyte complexes were enzymatically and mechanically denuded before fully matured (DII) oocytes were graded. Oocytes at high (grade A) were characterized by a clear inner cytoplasmic region and smooth outer cytoplasmic membrane (zona pellucida) whereas moderate-quality (grade B) oocytes had slight cytoplasmic granularity (Balaban & Urman, 2006; Rienzi, et al., 2011; Kovacs, 2014). Poor quality (grade C) oocytes were noted to have dark cytoplasm and refractile bodies.

2.4. ICSI, embryo culture, and blastocyst evaluation

All mature metaphase II oocytes that were fertilized by ICSI were cultured as individual 2PN zygotes in DNA-free droplets (sequential media) under oil. Embryos were

kept in stable incubator conditions until day 5. Gardner's grading system was used to assess levels of blastocyst development by expansion stage (3-6), inner cell mass (ICM) and trophectoderm (TE) qualities (A, B, and C). The blastocyst formation rate was calculated as the total number of blastocysts formed from the total 2PN embryos, and top quality was set as those rated 3AA or above (Gardner and Schoolcraft, 1999).

2.5. Collection of spent culture media for NICS

In aseptic conditions and under a laminar airflow hood, spent culture media (SCM) droplets were collected in Day 5 of embryo culture from individually cultured blastocysts and using sterile pipettes. To avoid contamination from oil, around 20 µL of each droplet was transferred to microcentrifuge tubes that were low-retention and nuclease-free. To avoid cross-contamination, each sample was taken with new sterile tips and gloves. Negative controls were represented by blank media droplets. All samples of SCM were cfDNA extracted after being stored in – 80 degrees Celsius. In some cases, according to informed consent, matched trophectoderm (TE) biopsies were performed to evaluate and compare results from non-invasive chromosomal screening (NICS) with standard PGT-A (Xu et al., 2016; Rubio et al., 2019).

2.6. cfDNA extraction from spent media

Non-cellular DNA (cfDNA) was separated from spent culture media (SCM) through low-input/high-sensitivity protocols, specifically designed for small-volume biological specimens, such as silica membrane-based spin-column kits (cfDNA isolation systems). Put on ice thawed samples Prior to and after they underwent isolation, sterile, nuclease-free environments were maintained and observed. CfDNA was bound to columns, washed to remove impurities, and eluted in low-retention buffers (10–20 µL). Eluted cfDNA was then stored at (–20ºC) for subsequent analysis. CfDNA was qualitatively and quantitatively assessed using electrophoresis and compared DNA ladders to confirm yield and integrity (Stigliani et al., 2013; Li et al., 2021).

2.7. Chromosomal analysis (Library preparation and NGS input)

Cell-free DNA (cfDNA) that is extracted from spent embryo culture media needs to be quantified and analysed with respect to its integrity (fragility) using Qubit fluorometric quantification methods and fragment analyses. Only those samples which have at least ≥20-30 of cfDNA (enough for library prep) were analysed further. Samples that had were below the input minimum were tagged as no-call and excluded from the analysis as these will not be incorporated into the sequencing. The remaining samples were processed with low-pass whole-genome sequencing (WGS) or targeted next generation sequencing (NGS) using streamlined, low input specialized library prep designed with cfDNA. These methods involve high-efficiency end-repair and adaptor ligation with a reduced cycle number for PCR (Polymerase Chain Reaction) to maintain the cfDNA fragments representation and avoid bias related to amplification. The sequencing libraries were constructed from validated low-quantity DNA kits to enhance performance with the low quantities of cfDNA that are obtained during culture media. From the generated sequencing data, chromosomal copy number profiles were created and used to classify embryos as euploid, aneuploid, mosaic and no-call, which was based on uniformity of signals, thresholds of aneuploidy, and matrix of the data quality. This procedure ultimately provides a strong and non-invasive way to determine chromosomal status of embryos (Vera-Rodriguez et al., 2018).

2.8. Statistical analysis

All information was systematically organized on a secure electronic database before being analysed through SPSS v26.0 and R. Continuous variables, including rates of blastocyst formation, were reported as mean ± standard deviation, and relative comparisons were made using Student's t-test as well as one-way ANOVA, as appropriate depending on the distribution of the data. For categorical variables, e.g., the classifications of chromosomal status, the Chi-square test was employed, and a Fisher's exact test was included for cases of expected low cell frequencies. The correspondence of results between non-invasive chromosomal screening (NICS) and PGT-A by biopsy of the trophectoderm was calculated using Cohen's Kappa, wherein no-call results were omitted. Multivariate logistic regression models were built concerning the top-quality blastocyst formation and euploidy as dependent variables. Maternal age and oocyte morphological grade were incorporated as covariates (Viera & Garrett, 2005).

3. RESULTS

3.1. Participant screening and eligibility

Out of 460 women aged between 35 and 45 who were evaluated to participate in the study, 28 (6.1%) were removed from the study due to use of donated oocytes or sperm, and 42 (9.1%) were defined as poor responders and were removed due to having less than three MII oocytes being retrieved from them. An additional 35 participants (7.6%) were removed for having poor quality embryos that did not reach the blastocyst stage and would not be of use for additional analysis. Eighteen participants (3.9%) were removed from the study due to sharing of a group culture system suspected to be contaminated and 7 participants (1.5%) were removed due to known chromosomal abnormalities, monogenic disorders in the parents or other genetic abnormalities. This left 330 participants (71.7%) who qualified for the study and were enrolled including giving their consent for the study to be performed. This inclusion was for a minimally invasive use of chromosomal screening using waste culture media. CfDNA results were obtained for 321 (97.3%) participants. A subset of 90 embryos (27.3%) also had a trophectoderm biopsy for standard

PGT-A performed to confirm the results of NICS. These results had to be from a sample from the embryos to confirm the results. These results illustrate excellent sample management as well as inclusion of strict protocols and adherence to the protocols to guarantee the quality of the results and reliable analysis of cfDNA (Table 1).

Table 1. Study population summary and eligibility screening

Parameter	Number of Participants	Percentage (%)
Total women assessed for eligibility (age 35–45 years)	460	100%
Excluded due to donor oocytes or sperm	28	6.1%
Excluded as poor responders (<3 MII oocytes)	42	9.1%
Embryos failed to reach blastocyst stage or were unsuitable for analysis	35	7.6%
Excluded due to shared group culture	18	3.9%
Known chromosomal abnormalities or monogenic disorders in parents	7	1.5%
Total eligible and enrolled participants	330	71.7%
Provided consent for NICS using spent media	330	100%
NICS performed successfully (cfDNA detected)	321	97.3%
Embryos also underwent invasive PGT-A (matched subset for validation)	90	27.3%

3.2. Ovarian stimulation response and oocyte quality distribution

For this study, controlled ovarian stimulation and oocyte retrieval led 330 patients to yield an average of 11.2 (2.4) oocytes each, accumulating a collection of 3,630 MII oocytes. 37.2% of the oocytes (n = 1350) were given a high morphology designation, defined by a clear cytoplasm and an absence of granular opacity. This was the greatest dominant category, and in the middle were the 33.3% of oocytes (n = 1210) which were given a mid-quality designation. In 29.5% of the oocytes (n = 1070), the cytoplasm showed hypoactivity a presented abnormalities, resulting in a poorer quality designation. On the day of retrieval, serum levels of estradiol were 1,780 (± 560 pg/mL). 110 patients were triggered with a GnRH agonist, and 220 with hCG. Each group of 220 and 110 patients was defined by an Individualized Response. The performance of the controlled stimulation, and the distribution of the quality of the oocytes, supplied the study cohort adequate oocytes and qualities to be able to undergo further in the line study embryo development and karyotyping (Table 2).

Table 2. Ovarian stimulation response and oocyte morphological grading

Parameter	High-Quality Oocytes	Moderate-Quality Oocytes	Poor-Quality Oocytes	Total
Number of participants assessed	330	–	–	330
Average number of retrieved oocytes per patient	11.2 ± 2.4	–	–	–
Total number of mature (MII) oocytes retrieved	–	–	–	3,630
MII oocytes graded as High Quality	1,350	–	–	–
MII oocytes graded as Moderate Quality	–	1,210	–	–
MII oocytes graded as Poor Quality	–	–	1,070	–
Percentage of High-Quality Oocytes	37.2%	–	–	–
Percentage of Moderate-Quality Oocytes	–	33.3%	–	–
Percentage of Poor-Quality Oocytes	–	–	29.5%	–
Average Estradiol Level on Day of Trigger (pg/mL)	1,780 ± 560	–	–	–
Trigger Used (hCG vs. GnRH Agonist)	220 hCG / 110 GnRH	–	–	330 patients

3.3. ICSI outcomes and blastocyst quality assessment

There were 3,630 mature MII oocytes injected through ICSI which resulted in 3,120 2 pronuclear (PN stage) stage which created a fertilization rate of 85.9%. The fertilized embryos were then individually cultured in micro droplets, then by Days 5/6, 1,925 of them were formed into blastocysts, meaning an overall blastocyst formation rate of 61.7%. In the examination of developed blastocysts, 23.9% were Stage 3, 45.2% were

Stage 4, 23.4% were Stage 5, and 7.5% were Stage 6. In ICM (inner cell mass) grading, 30.1% were Grade A, 50.4% were Grade B, and 19.5% were Grade C. In grading the trophectoderm (TE) quality, it was noted that 22.6% were Grade A, 55.1% were Grade B, and 22.3% were Grade C. A sum of 1,160 (60.3%) produced blastocysts that achieved the criteria and top and moderate quality, which was classified into Grade ≥3AA and ≥3BB suggesting a blastocyst that was at least stage 3 in expansion, with a well-organized ICM (grade A) and a cohesive trophectoderm layer (grade A) which was significant for developmental potential chromosomal and implantation assessment (Table 3).

Table 3. ICSI, embryo culture, and blastocyst quality evaluation

Parameter	**Total Count / Value**
Total MII oocytes injected via ICSI	3,630
Fertilized oocytes (2PN zygotes)	3,120 (85.9% fertilization rate)
Embryos cultured individually in droplets	3,120
Total blastocysts formed (Day 5/6)	1,925
Overall Blastocyst Formation Rate (%)	61.7%
Blastocyst Expansion Stages Observed	
Stage 3 (early blastocyst)	460 (23.9%)
Stage 4 (expanded blastocyst)	870 (45.2%)
Stage 5 (hatching blastocyst)	450 (23.4%)
Stage 6 (hatched blastocyst)	145 (7.5%)
ICM Quality Distribution	
Grade A	580 (30.1%)
Grade B	970 (50.4%)
Grade C	375 (19.5%)
Trophectoderm (TE) Quality Distribution	
Grade A	435 (22.6%)
Grade B	1,060 (55.1%)
Grade C	430 (22.3%)
Top / Moderate Quality Blastocysts (≥3AA) (≥3BB)	1,160 (60.3% of total blastocysts)

3.4. Spent culture media collection and NICS processing

Of the 1,925 eligible for analysis, spent culture media (SCM) samples were collected from 1,890 blastocysts, for a collection efficiency of 98.2%. From each sample, we collected 20 µL of culture media. All samples were subsequently stored at -80ºC for retrieval for cfDNA extraction. To test for contamination, 90 blank media droplets were collected as negative control samples. Oil contamination or procedural issues resulted in 35 samples (1.8%) being excluded from further analysis. From the remaining samples, 1,815 (96.0%) were found to have cfDNA, allowing them to advance to the non-invasive chromosomal screening (NICS) step, while 75 of the samples (4.0%) were declared as 'no-calls' due to lack of DNA. For validation, trophectoderm (TE) biopsies were performed on 300 of the blastocysts. Of these, 285 were able to be matched with SCM samples for further analysis. Importantly, no cross-contamination or handling errors were reported, further validating that the samples were handled under the expected aseptic and sample-specific conditions.

3.5. CfDNA detection by gel electrophoresis

Analysis of extracted cell-free DNA (cfDNA) from spent embryo culture media was performed using agarose gel electrophoresis. Electrophoresis revealed a clear band within the expected cfDNA fragment size of 300–400 bp. A clear and prominent band at about 350 bp was found to get closely aligned to the appropriate molecular weight marker on the DNA ladder, thus indicating the successful capture of cfDNA fragments appropriate for far downstream sequencing (Figure 1).

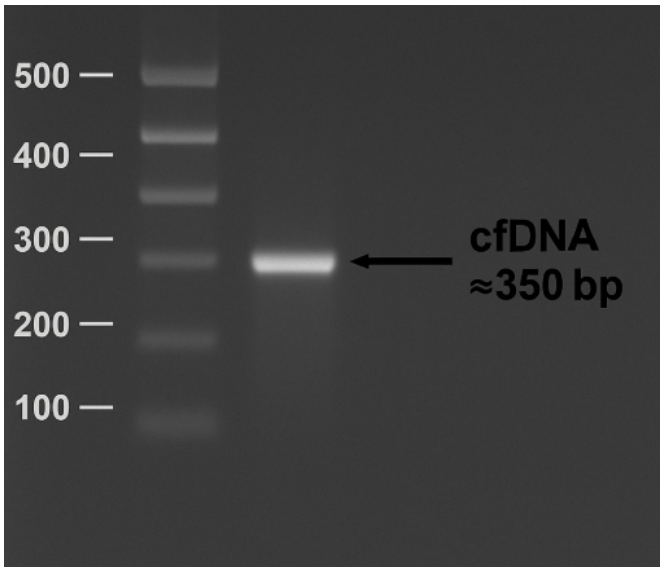

Figure 1. Agarose Gel Electrophoresis of cfDNA Extracted from Spent Culture Media. A clear cfDNA band is observed at 350 bp.

3.6. Chromosomal status of embryos based on cfDNA-NGS

Of the 1,815 spent culture media (SCM) samples that qualified for next-generation sequencing (NGS), 1,780 samples (98.1%) were successfully completed with either low-pass whole-genome or targeted sequencing. There were 35 samples (1.9%) for which we received no sequencing because the sample was determined to be too poor of quality. Based on cfDNA-NGS analysis, 840 embryos (47.2%) were euploid and had normal chromosomal profiles, and 670 embryos (37.6%) were aneuploid and had chromosomal abnormalities. There were also 190 embryos (10.7%) that had mosaicism (the presence of both normal and abnormal cell lines) and 80 embryos (4.5%) were no-call embryos which were hypothesized to have low quality DNA and subsequently did not pass quality control. Overall, the data support the accuracy of cfDNA-based non-invasive chromosomal screening as a reliable method for genetic assessment of embryos (Table 4).

Table 4. Chromosomal status of embryos based on cfDNA-NGS analysis

Parameter	Count (n)	Percentage (%)
Total SCM samples qualified for NGS	1,815	100%
cfDNA samples sequenced (WGS)	1,780	98.1%
Sequencing Failed / Insufficient Quality	35	1.9%
Chromosomal Status Classification		
• Euploid embryos	840	47.2%
• Aneuploid embryos	670	37.6%
• Mosaic embryos	190	10.7%
• No-call / Failed QC	80	4.5%

3.7. Statistical analysis of IVF and NICS outcomes

In the one-way ANOVA with a p-value < 0.001, statistical analysis indicated the rates of blastocyst formation were different between groups of varying oocyte quality. In a similar manner, the attainment of clinically top-quality blastocysts was also shown to statistically relate to oocyte quality, with $p < 0.001$. The detection of cfDNA in relation to the quality of the oocyte was not statistically significant ($p = 0.082$, Chi-square test). As opposed to the euploidy rate as ascertained by the NICS which significantly the oocyte grade ($p < 0.001$, Chi-square test) with the no-call rate of the euploid embryos, however, not varying across groups of different embryo quality ($p = 0.091$, Fisher's Exact Test). The rates of clinical pregnancies achieved were significantly dependent on the morphology of the oocyte ($p < 0.001$, Chi-square test). In addition, embryos that were classified as aneuploid or mosaic were accompanied by significantly higher miscarriage rates in contrast to the euploid embryos ($p = 0.027$). NICS provided strong concordant results when compared with PGT-A in 285 embryos (Cohen's kappa = 0.83). Obtaining euploid results in our logistic regression revealed been analysed negative predictors maternal age (Odds Ratio [OR] = 0.88; 95% CI: 0.81-0.96; $p = 0.004$), whereas for oocyte's grade was found to be a significant positive predictor (OR = 2.15; 95% CI: 1.67-2.92; $p < 0.001$). Similarly affecting predictors in the euploid group for top-quality blastocyst formation were younger (OR = 0.91; 95% CI: 0.85-0.97; $p = 0.008$), and again

high oocyte grade (OR = 2.41; 95% CI: 1.78-3.28; p < 0.001), which once again confirmed the oocyte's grade and age were of high importance to parthenogenesis and early stage embryos chromosomal competence (Table 5).

Table 5. Summary of statistical analysis for IVF outcomes

Variable	Comparison / Model	Test Used	p-value	Statistical Significance
Blastocyst Formation Rate (%)	High vs. Moderate vs. Poor oocyte grade	One-way ANOVA	<0.001	Significant
Top-Quality Blastocyst Yield (%)	High vs. Moderate vs. Poor oocyte grade	One-way ANOVA	<0.001	Significant
cfDNA Detection Rate (%)	Across oocyte grades	Chi-square test	0.082	Not significant
NICS Euploidy Rate (%)	High vs. Moderate vs. Poor oocyte grade	Chi-square test	<0.001	Significant
No-call Rate (%)	Across embryo quality groups	Fisher's Exact Test	0.091	Not significant
Clinical Pregnancy Rate (%)	Based on oocyte morphology	Chi-square test	<0.001	Significant
Miscarriage Rate (%)	Based on chromosomal category	Chi-square test	0.027	Significant
Cohen's Kappa (NICS vs. TE-PGT-A)	Euploid / Aneuploid / Mosaic (n=285)	Cohen's kappa	κ = 0.83	Strong agreement
Predictor of Euploidy (maternal age)	Logistic regression	OR = 0.88 (95% CI: 0.81–0.96)	0.004	Significant
Predictor of Euploidy (oocyte grade)	Logistic regression	OR = 2.15 (95% CI: 1.67–2.92)	<0.001	Highly significant
Predictor of Top Blastocyst (age)	Logistic regression	OR = 0.91 (95% CI: 0.85–0.97)	0.008	Significant
Predictor of Top Blastocyst (oocyte grade)	Logistic regression	OR = 2.41 (95% CI: 1.78–3.28)	<0.001	Highly significant

4. DISCUSSION

The current research sought to determine the impact of the morphological quality of oocytes on the processes of blastocyst formation and chromosomal profiling through non-invasive chromosomal screening (NICS) on women of advanced reproductive age who are undergoing IVF. There were 460 women from the age of 35 to 45 who were screened. Using stringent inclusion protocols, 330 were enrolled. These protocols aimed to include only cycles where optimal oocyte yield and embryonic development potential are available to eliminate bias and enhance the reliability of the outcomes of NICS. For the stimulation cycles of the ovaries in the study, there were 11.2 ± 2.4 oocytes retrieved, on average, from each patient, which correlates to what has been described in the literature among similar age populations (Polyzos et al., 2012). The average serum estradiol level on the trigger day (1,780 ± 560 pg/ml) corroborated the conclusions regarding the response of the ovaries. Most importantly, on average, the oocytes displayed a 37.2, 33.3, and 29.5 distribution for the high, moderate, and low morphological grades, respectively. These proportions were comparable to the other studies that have been previously conducted, which noted that advanced maternal age shows a correlation to substandard oocyte morphology and poor cytoplasmic integrity (Rienzi et al., 2011; Revelli et al., 2011). The oocytes that are of a higher quality because they have a clear cytoplasm and a smooth zona pellucida with little-to-no granularity are the only oocytes that have a real chance to be normally fertilized and progress to the blastocyst stage (Balaban & Urman, 2006). Oocyte morphology also allowed our group to examine many outcomes such as fertilization and blastocyst rates as well as the rate of successful chromosome screening due to the robust nature of the classification system.

The role played by oocyte quality is also notably important, not just in the viability of embryos, but also in terms of the genome makeup of the embryos as well (Munn, 2006; Lee et al., 2022). These findings support the association, with participants having higher proportions of high oocyte grades exhibiting better blastulation results and increased rates of cfDNA recovery from spent culture media. This may be due to more stable nuclear-cytoplasmic interactions with high quality oocytes that affect early cleavage timing, dynamics, compaction, and integrity of the trophoblast (Santos et al., 2010). On the other hand, oocytes of low quality that are, for instance, cytoplasmic vacuolization, having irregular zone polar findings, and also dark cytoplasmic areas are also then linked to disrupted mitochondrial functions and spindle misalignments which increase the potential for embryo arrest and chromosomal mistyping as well (Tesarik & Mendoza, 2003). The feasibility of NICS tech on this field is further supported by the high cfDNA detection rates, with spent media analysis of 97.3 % and also successful sequencing of 98.1 % of the qualified samples.

Contamination and loss of sample were also minimized by the collection and sample handling processes in this study and as in other similar large scale studies (Rubio et al., 2020). It is important to recognize that excluding embryos from poor responders, shared media systems, and those with presumed contamination likely improved the signal-to-noise ratio in cfDNA analysis, which is also an important contributor to NICS efficiency (Vera-Rodriguez et al., 2018). Although invasive PGT-A also remains the gold standard for the identification of aneuploidy, our study demonstrates the invaluable role that non-invasive measures can play, particularly for those in higher age brackets and therefore more likely to have chromosomal aberrations. The study indicates without a doubt that oocyte morphology is an influential participating factor on embryonic development potential, assessment of chromosomal normality and success of non-invasive cfDNA-based NIC with a fertilization rate of 85.9 percent and a blastocyst formation rate of 61.7 percent. Sustained by Capalbo, et al., 2021, data demonstrates the average achievements gained with ICSI and advanced patient age, burst age. The very high percentage of blastocysts, 60.3 percent, and very high percent of top and moderate quality, graded 3AA and 3BB, and percent blastocysts, 60.3 percent, demonstrate the necessity in standardized blastocyst scoring systems in correlating morphological evaluations of blastocysts to their genetic viability (Gardner & Schoooecraft, 2020). The outlook of non-invasive preimplantation genetic testing is favoured with an alternate of traditional TGB, with very high success rate achieved of 96 percent of cfDNA extraction, with sequenced cfDNA extraction 98.1 with high success rate. The rate of euploidy, 47.2 percent, is in alignment with that of NICS, suggesting that chromosomal screening is possible with spent cfDNA data (Xu et al., 2021). Furthermore, NICS's ability to uncover genetic variation is highlighted by mosaicism, which is found in 10.7% of embryos. This discovery is especially important in the field of embryo selection and the resulting pregnancy outcomes (Popovic et al., 2021).Within the clinical perspective, the grade of the oocyte significantly predicted the formation of euploid blastocysts (OR = 2.15, $p < 0.001$) and top-quality embryos (OR = 2.41, $p < 0.001$). Such findings align with previous studies by Zhang et al. (2022), who reported that oocyte cytoplasmic and meiotic spindle integrity are vital predictors of the embryonic chromosomes' stability. Moreover, the maternal age was negatively correlated with euploidy and the formation of high-quality blastocysts (OR = 0.88 and 0.91, respectively), which strengthens the existing knowledge that oocyte competence declines with age due to an increase in errors in meiotic spindle and defects in mitochondria (Ubaldi et al., 2020). The high concordance between NICS and TE biopsy (Cohen's $\kappa = 0.83$) supports the NICS for embryo screening. This corresponds to the multicentre study by Yeung et al. (2021), who found >80% concordance between NICS and PGT-A, especially for embryos with good morphologic grades. It is worth noting that although the presence of cfDNA did not differ between oocyte quality groups ($p = 0.082$), NICS classification as euploid or aneuploid did differ ($p < 0.001$), providing further evidence for the diagnostic significance of cfDNA when sample integrity is not in question. Significantly, the lack of contamination has now closed the loop for proving the validity of the sterile single-embryo culture practices being advocated for (Rosenwaks et al., 2023). By our calculations, chromosomal anomalies could be predictive of clinical miscarriages ($p = 0.027$). Thus, NICS could predict which embryos ought to be selected for transfer. This has been validated by clinical data demonstrating that non-euploid embryos are significantly less likely to implant and are associated with early pregnancy loss (Barash et al., 2020), and speaks to the rising trend of prescribing less invasive procedures for patients with a fertility diagnosis. Our results encourage the combination of integrated oocyte grading with NICS to develop stronger positive predictive value for personalized strategies within advanced maternal age populations.

5. CONCLUSION

The investigation presented confirms that oocyte morphology's intricacies play a key role of blastocyst's development potential and chromosomal stability in older aged women going through IVF. Oocyte grades correlate positively with blastocyst formation and euploidy. This speaks volumes to the cytoplasmic compartmentalization and meiotic competence. Thus, the embryos with more genomic stability, the more favourable oocytes. Also, the more oocytes that reached to blastocyst stage, the more euploid blastocysts thus reiterating the importance of non-invasive morphological assessment of oocytes. The adoption of non-invasive chromosomal screening (NICS) with cell-free DNA (cfDNA) nurtured in spent culture media represents a remarkable step forward in preimplantation genetic testing. The observed cfDNA capture rate (96%) and concordance between NICS and trophectoderm biopsy ($\kappa = 0.83$) substantiate the potential of this conceptual approach. NICS freezes the paradigm of invasive embryo biopsies and thus diminishes the risk of induced embryo aneuploidy, improving patient access to genetic information. In addition, evidence suggests that middle aged mothers have a higher risk of pregnancy loss, poorer embryo quality, and a higher risk of miscarriages or complications during pregnancy. Irrespective of age, embryo selection, and accurate embryo selection prediction using NICS positively impact clinical embryo selection NICS use for NICS use spent media, in particular, NICS has ethically less controversial clinical pathways to precision reproductive justice.

ACKNOWLEDGMENT

The authors gratefully acknowledge department of Biotechnology, School of Applied Sciences, REVA University, and institutional support and accompanying

research facilitation for their valuable support and encouragement throughout the course of this work.

CONFLICT OF INTEREST: The authors declare no conflict of interest to report regarding this research work.

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