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Cell-free fetal DNA versus maternal serum screening for trisomy 21 in pregnant women with and without assisted reproduction technology: a prospective interventional study

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Purpose: Cell-free DNA (cfDNA) as a primary screening test has been available for years but few studies have addressed this option in a prospective manner. The question is of interest after reports that maternal serum screening (MSS) is less accurate for pregnancies resulting from assisted reproduction technologies (ART) than for spontaneous pregnancies (SP).

Methods: A prospective interventional study was designed to address the performances of cfDNA compared with MSS in pregnancies with or without ART. Each patient was offered both MSS and cfDNA testing. The primary analysis cohort ultimately included 794 patients with a spontaneous pregnancy (SP) (n = 472) or pregnancy obtained after ART (n = 322).

Results: Overall, the false-positive rate and positive predictive value were 6.6% and 8.8% for MSS but 0% and 100% for cfDNA. MSS false-positive rate and positive predictive values were clearly poorer in the ART group (11.7% and 2.6%) than in the SP group (3.2% and 21.1%). The global rates of invasive procedures were 1.9% (15/794) with cfDNA but 8.4% (65/794) if MSS alone was proposed.

Conclusion: cfDNA achieved better performance than MSS in both spontaneous and ART pregnancies, thus decreasing the number of invasive procedures. Our findings suggest that cfDNA should be considered for primary screening, especially in pregnancies obtained after ART.

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Key Words: assisted reproduction technology; cell-free fetal DNA; first trimester; maternal serum screening; trisomy 21

INTRODUCTION

Screening for Down syndrome is offered to pregnant women in numerous countries. The strategy typically considered the most efficient is first-trimester screening between 11 and 13^{+6} weeks, in a combined evaluation of maternal age, serum markers, and fetal nuchal translucency thickness. Above a defined threshold, which varies by country, invasive sampling is proposed to patients to determine whether the fetus is affected, establishing a standard or molecular fetal karyotype. First-trimester combined screening has a poor positive predictive value (PPV), estimated at approximately 6%,[1] and although invasive sampling has recently been reported as carrying low risk of miscarriage,[2] these risks are still inherent to the procedure.

Cell-free fetal DNA (cfDNA) testing has spread rapidly since 2011 for screening in clinical practice.[3] The high performance of cfDNA assays in screening for trisomy 21 (T21), and to a lesser degree for trisomies 18 and 13, has encouraged many scientific societies to publish committee opinions and guidelines regarding the conditions and use of cfDNA in pregnant women for fetal aneuploidy.[4–6] The growing experience of clinicians combined with tests conducted by providers has yielded false-negative and false-positive cfDNA results. Vanishing twins, confined placenta mosaicism, and maternal mosaicism or malignancies have been described as possible sources of discrepancies, with the fetal karyotype confirming the screening but not the diagnostic value of cfDNA. Despite these rare cases, cfDNA testing has been considered a first-tier screening test, but only a few studies have assessed its performance.[3,7,8] All showed that cfDNA had higher PPVs for T21 detection than standard

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screening. However, none of these studies were conducted in a prospective and interventional manner, meaning the real impact of implementing cfDNA testing in clinical practice was not demonstrated. In addition, these studies often combined screening during the first, second, and/or third trimesters, reported a high number of no-call results, and did not address complete follow-up or pregnancy issues. Despite the exceptional performance of cfDNA testing, the most recent recommendations (i.e., from the Society for Maternal–Fetal Medicine) do not advise its use as a primary screening option because several questions remain unanswered.[5] The most relevant and interesting of these questions are (i) the exact number of no-call results and (ii) the number of patients who will undergo invasive sampling due to abnormal ultrasound examination at follow-up or maternal anxiety, even when classed as lower risk after screening. Maternal anxiety might be of particular importance in a population of women achieving pregnancy via assisted reproduction technology (ART),[9] for which most studies found combined screening in the first trimester to be less accurate, along with a possible increase in the number of high-risk patients due to modification of pregnancy-associated plasma protein A (PAPP-A) and human chorionic gonadotropin (hCG) levels.[10] Because these modifications might reflect impaired placentation and be associated with obstetrical complications and/or adverse neonatal outcomes such as hypertension, preeclampsia, or intrauterine growth restriction,[11–13] one can hypothesize that fetal DNA release (fetal fraction (FF)) into maternal circulation might also be affected and cfDNA analysis performance modified.[14] The literature is contradictory on that matter; some groups reported no significant contribution of method of conception[15,16] while others observed a decreased FF in pregnancies conceived by in vitro fertilization (IVF).[17] Additionally, the higher prevalence of vanishing twins in ART pregnancies[18] might impact the performance of cfDNA screening through a higher rate of false-positive results.

We therefore designed a prospective, interventional, multicenter study to assess the real-life performance and impact of cfDNA-based screening compared with standard maternal serum screening (MSS) as primary screening in singleton pregnancies and to compare their performance in pregnancies achieved with and without ART.

MATERIALS AND METHODS

Patients and data collection

From May 2015 to February 2016, pregnant women undergoing aneuploidy screening in nine French centers were enrolled. Of these centers, two were maternity units connected to a referral center for ART to collect a sufficient number of cases of pregnancies achieved by ART (IVF, intracytoplasmic sperm injection (ICSI), or oocyte and egg donation).

The institutional review board (Comité de Protection des Personnes) approved the study protocol (CPP no. 14–054), with written informed consent obtained from all patients. All patients had planned and agreed to first- or second-trimester screening, which includes an ultrasound scan for nuchal translucency measurement, performed by a certified (by national quality-review programs) sonographer and, when ultrasounds were normal, blood samples for both conventional MSS and cfDNA testing. Both sets of test results were returned to the practitioner in charge of the study at the local site then to the patient when received. Patients exhibiting fetal anomalies on the first-trimester scan (including nuchal translucency ≥ 3.5 mm) were excluded from the study as cfDNA is not recommended in this case.[19] The local site teams monitored all pregnancies. A research assistant collected all clinical data into the electronic case report form, Cleanweb (Telemedecine Technologies, Boulogne-Billancourt, France). Clinical monitors verified the data through a review of source documents.

Clinical outcomes

The practitioners first communicated the standard screening results to the patients. Due to their longer technical requirements, cfDNA test results were available a few days later. During precounseling, the patients were informed that their results would be communicated in two stages. In cases where a patient was classified into a high-risk group following MSS (risk score $\geq 1/250$ was considered high, according to French recommendations), they were offered either to immediately undergo invasive sampling or to wait for the cfDNA report. No risk score was calculated for trisomy 18 or 13, as this is not routine practice in France. For each patient, all laboratory and ultrasound reports (usually first, second, and third trimester, according to French recommendations), invasive procedures (if performed), cytogenetic testing reports, and pregnancy outcomes were recorded. In the event of miscarriage, termination of pregnancy, or in utero demise, fetal karyotype was performed where possible. For all live births, local pediatricians performed newborn physical examinations on site and sent for genetic testing if required. When examinations were normal, the newborns were considered unaffected, even if no genetic testing was performed.

Cell-free fetal DNA analysis in maternal plasma

The cfDNA was performed by massive parallel sequencing using a whole-genome approach, as described by Jensen et al.[20] with some slight modifications. Maternal blood was collected in two cfDNA BCT Streck tubes. Plasma was isolated within 4 days of collection by a double centrifugation procedure. Samples were rejected if the tubes were broken on arrival or not sufficiently filled. Total DNA was extracted from 4 mL of plasma by means of the QIAamp DSP Circulating Nucleic Acid Kit (Qiagen, Courtaboeuf, France) after thawing and centrifugation of the samples, then eluted in 55 µL of elution buffer, in accordance with manufacturer's instructions. The DNA libraries were then prepared starting from 50 µL of extracted DNA solution using the NEBNext Ultra DNA Library Prep Kit (NEB, Evry, France). After quantification on the LabChip GX microfluidic platform

(Perkin-Elmer, Courtaboeuf, France), the libraries from 12 different samples were pooled and sequenced on each lane of an Illumina V3 flow-cell on a HiSeq1500 instrument, using the Truseq SBS kit V3-HS reagent (Illumina, Paris, France) for 27 cycles. Finally, the sequence reads were mapped to the University of California–Santa Cruz hg19 version of the human genome using Bowtie version 2; *Z*-scores were calculated for the targeted chromosomes 13, 18, and 21, as previously described;[20] and the FF was evaluated using the coverage method, as described by Kim et al.[21] The results were expressed as "positive" or "negative" according to the following metric criteria: total count ≥ 9 million and estimated fetal DNA fraction $\geq 4\%$. The latter metric is of particular relevance, as it may influence test performance for cfDNA assays based on counting methods. A 4% value is often used as a cutoff, based on the demonstration by Fan and Quake.[22] The classification relied on a standard normal transformed cutoff value of $z = 3$ for chromosome 21 and $z = 3.95$ for chromosomes 18 and 13.[20]

Study outcomes

The study's primary outcome was to compare the false-positive rate and PPV of standard MSS with those of cfDNA for T21 in their use as primary screening tests in a general population of pregnant women with spontaneous or ART-achieved pregnancy. Secondary outcomes included the percentage of invasive procedures induced by abnormal findings on ultrasound follow-up or for any other reason such as maternal anxiety, the rate of "no-call" results, and the turnaround time for cfDNA reports. We also intended to evaluate cfDNA's ability to assess the risk of trisomies 18 and 13.

Statistical analysis

The database was validated and locked before the statistical analysis. Descriptive results were displayed as percentages for categorical variables, and median and interquartile range (IQR) for quantitative variables. The efficiency was characterized in terms of false-positive rate (or specificity), false-negative rate, and predictive positive value. Exact 95% confidence intervals (CIs) were computed with the binomial distribution. False-positive and false-negative rates of cfDNA and standard MSS were compared using McNemar's chi-square test. Raw and adjusted differences in the false-positive rates for serum screening between spontaneous (SP) and ART pregnancies were estimated using logistic regression. The number of included subjects was computed to be able to detect with a statistical power of 90% a difference of 3.5% between the false-positive rates of cfDNA and standard MSS. Allowing for a 15% loss to follow-up or missing data rate, 500 subjects were required in the SP and ART groups. Analyses were done with Stata 14 software (Stata Statistical Software, release 13; Stata, College Station, TX).

RESULTS

Patient data

A total of 924 patients with singleton pregnancies were enrolled in nine French centers (546 with spontaneous pregnancies; 378 with ART-induced pregnancies). To analyze the data concerning best clinical practices and thus avoid overinterpretation, we decided to initially focus on patients who received MSS during the first trimester. Consequently, 111 cases were excluded from the global analysis due to MSS and/or cfDNA being performed during the second trimester. The data from 24 other patients were also excluded due to either MSS or cfDNA screening being performed but not both ($n = 22$), the patient being lost to follow-up ($n = 1$), or the samples not fully respecting preanalytical requirements ($n = 1$). For the last patient, no results were available for either screening test because the blood tubes were broken during transportation. This patient did not accept a second sampling and gave birth to healthy infant. The primary analysis cohort finally included 789 patients with either SP ($n = 469$) or ART-induced pregnancies ($n = 320$) (**Figure 1**). The demographic and clinical characteristics of patients are shown in **Table 1**. All women underwent typical follow-up during their pregnancy until delivery, according to French best practice guidelines and regulations.

Primary analysis

Primary data analysis covered the 789 patients who accepted blood sampling for concurrent standard MSS and cfDNA primary screening for trisomy 21 during the first trimester of pregnancy and received both reports.

All standard MSS results were available within a mean turnaround time of 1 day (1–3), with a mean wait for cfDNA results of 10 days (9–12). Four patients had to be resampled because the initial cfDNA assay was inconclusive. For three of them, the final result was reported, while the assay remained inconclusive in the fourth. The cfDNA analysis was attempted three times for this latter patient, at 12, 15, and 28 weeks, respectively, yet proved unsuccessful as the measured FF always remained below the required quality metrics ($< 4\%$). This patient with a body mass index (BMI) of 23 exhibited an antiphospholipid syndrome and was under heparin treatment. However, she did not opt for an invasive procedure as her initial risk was calculated at $< 1/10{,}000$ following standard MSS. Her baby was clinically normal at birth.

The comparative results of the two approaches for T21 screening are illustrated in **Figure 2**, with the data summarized in **Table 2**. Overall, T21 screening performance was much better using cfDNA than standard MSS. The false-positive rates and PPVs were 6.6% (95% CI, 5–8.6%) and 8.8% (95% CI, 2.9–19.3%) for MSS versus 0% (95% CI, 0–0.47%) and 100% (95% CI, 59.0–100%) for cfDNA. Five patients tested positive and chose to undergo invasive testing. All fetuses were confirmed to be affected after invasive procedures (amniocentesis $n = 4$; chorionic villus sampling $n = 3$) and fetal karyotyping, with one fetus carrying a mosaicism

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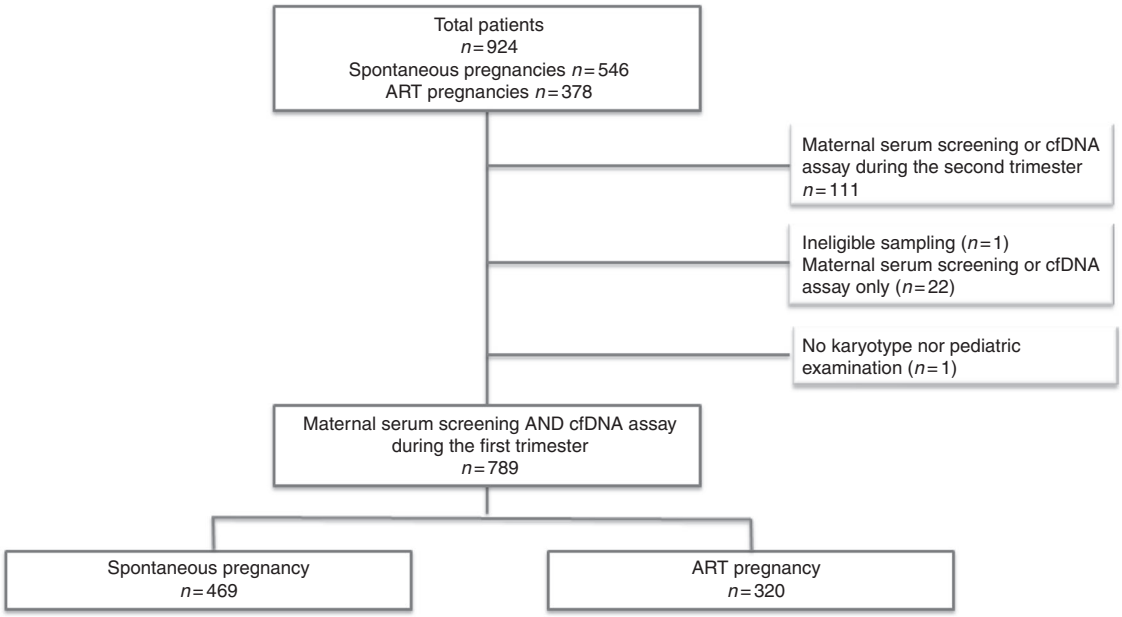

Figure 1 Enrollment of patients for primary analysis. ART, assisted reproduction technologies; cfDNA, cell-free DNA.

Table 1 Demographics and clinical characteristics of the patients

	All ($N=789$)	Spontaneous pregnancy ($n=469$)	ART pregnancy ($n=320$)
Age (years)	33.3 (30.0–37.5)	32.1 (29.0–35.5)	34.8 (31.5–39.5)
Age > 35 years	36.6%	28.4%	48.8%
BMI	22.5 (20.6–25.7)	22.3 (20.4–25.5)	22.6 (20.8–25.8)
Multiparous	45.1%	54.4%	31.7%
Mode of conception			
Spontaneous	59.4%	100%	0%
IVF	10.1%	0%	25.0%
ICSI	13.9%	0%	34.4%
Oocytes or embryo donation	3.8%	0%	9.4%
Frozen embryo	6.0%	0%	14.7%
Insemination	6.7%		16.6%
Gestational age at sampling	12^{+4} (12^{+2}–13^{+1})	12^{+4} (12^{+2}–13^{+1})	12^{+3} (12^{+1}–13)

Data shown as median and IQR values. ART, assisted reproduction technology; BMI, body mass index; ICSI, intracytoplasmic sperm injection; IQR, interquartile range (25th – 75th percentile); IVF, in vitro fertilization.

(mos47,XY,+21[3]/46,XY[23]). All patients chose to terminate pregnancy except the mother with a fetus carrying mosaicism. Two patients had a false-negative screening result with the standard procedure, while cfDNA assay was positive. Their risk scores were 1/652 and 1/531 with MSS, and nuchal translucencies were normal. Both patients underwent invasive testing, and the fetuses were confirmed to be affected with T21. Following standard MSS, 52 pregnant women were classified as high-risk patients, yet produced negative cfDNA assays. None chose to undergo the invasive procedure when their MSS results were reported, opting to wait for cfDNA results for the final decision. No invasive testing was carried out in this group, and all babies were healthy at birth. Finally, 730 patients were classified as low risk with MSS and negative for cfDNA assay as well. In this group, amniocentesis was conducted for eight patients due to one polymalformative syndrome revealed on ultrasound during the second trimester ($n=1$), one toxoplasmosis seroconversion with ventricular dilatation ($n=1$), two spina bifida ($n=2$), one severe hypospadias ($n=1$), one intrauterine death ($n=1$), one small for gestational age fetus with clinodactyly ($n=1$), and one small for gestational age with suspected cytomegalovirus infection ($n=1$). No invasive procedure was opted for based solely on maternal anxiety. Finally, the introduction of cfDNA reduced the number of invasive procedures by 78.5% (1.9% (15/789) vs. 8.2% (65/789) for MSS).

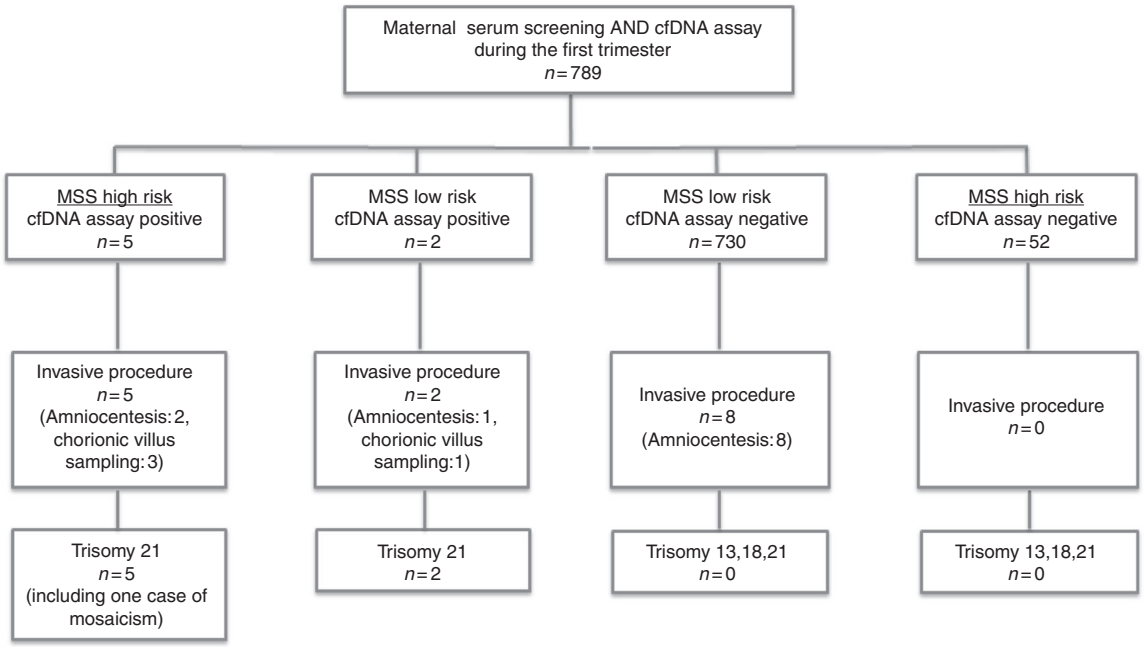

Figure 2 Outcomes for primary analysis. cfDNA, cell-free DNA; MSS, maternal serum screening.

Table 2 Maternal serum screening and cfDNA assay performance for a primary screening for trisomy 21 in both the spontaneous and ART pregnancy groups

All	**Maternal serum screening**	**cfDNA assay**	*p*
False positive	6.6% (5.0–8.6%)	0% (0–0.47%)	$p<1‰$
Specificity	93.4% (91.4–95.0%)	100% (99.5–100%)	$p<1‰$
False negative	28.6% (3.7–71.0%)	0% (0–41.0%)	$p=0.50$
PPV	8.8% (2.9–19.3%)	100% (59.0–100%)	NC
Spontaneous pregnancies	**Maternal serum screening**	**cfDNA assay**	***p***
False positive	3.2% (1.8–5.3%)	0% (0–0.79%)	$p<1‰$
Specificity	96.8% (94.7–98.2%)	100% (99.2–100%)	$p<1‰$
False negative	0% (0–60.2%)	0% (0–60.2%)	$p=1$
PPV	21.1% (6.1–45.6%)	100% (39.8–100%)	NC
ART pregnancies	**Maternal serum screening**	**cfDNA assay**	***p***
False positive	11.7% (8.4–15.7%)	0% (0–1.2%)	$p<1‰$
Specificity	88.3% (84.3–91.6%)	100% (98.8–100%)	$p<1‰$
False negative	66.7% (9.4–99.2%)	0% (0–70.8%)	$p=0.5$
PPV	2.6% (0.07–13.8%)	100% (29.2–100%)	NC

Data shown as median and IQR values. ART, assisted reproduction technology; BMI, body mass index; cfDNA, cell-free DNA; IQR, interquartile range (25th – 75th percentile); NC, not comparable; PPV, positive predictive value.

The mean FF was 10.6% (± 2.9). No correlation was found between FF and gestational age at sampling between 12+4 and 13+6 weeks, although BMI and FF were negatively correlated, as reported elsewhere.[23] Because the mean maternal age at screening among our cohort was higher than that typically observed in France (33.3 years (29.9–37.5)), comparisons were additionally made with a subgroup of 504 patients under 35 years old. In this subgroup, the mean maternal age was 30.7 (28.5–33.0), mean BMI 22.2 (20.4–25.5), and mean gestational age at sampling 12^{+5} (12^{+1}–13). Only one patient carried a confirmed T21-affected fetus; screening was positive with cfDNA but negative with MSS. Due to the small numbers, only specificities could be calculated: 97.4% (95% CI, 95.6–98.6%) for MSS and 100% (95% CI, 99.3–100%) for cfDNA ($p<1‰$).

Secondary analyses

When the two groups—SP and ART—were considered, the maternal age at screening significantly differed between them ($p<1‰$), with the patients undergoing ART typically older

(**Table 1**). The ART procedures were intrauterine insemination, 19.4%; IVF, 29.7%; ICSI, 37.0%; frozen embryo, 13.3%; egg donation, 0.6%. BMI did not significantly differ between the groups (22.1 (20.2 – 25.3) and 22.4 (20.8 – 25.9) ($p = 0.83$)), and gestational age at sampling was also similar, although the 2-day difference was statistically significant ($p = 0.01$). MSS screening performance in the ART group was less favorable, with a false-positive rate of 11.7% (95% CI, 8.4–15.7) and a PPV of 2.6% (95% CI, 0.07–13.8), especially when compared with cfDNA (0% (95% CI, 0–1.1%) and 100% (95% CI, 29.2–100%)), respectively (**Table 2**).

There was no difference in cfDNA-based screening performance for T21 between the SP and ART groups, where four and three fetuses were diagnosed as affected, respectively. This could be at least partially due to the medians of FF, respectively 10.9% (± 3.14) and 10.2% (± 2.7) in the SP and ART groups, being highly similar yet also statistically different ($p < 0.05$). Adjustment for gestational age at sampling and BMI did not modify the results. Interestingly, the proportion of patients with high FF (>15%) was smaller in the ART group ($p = 0.002$) (**Table 3**).

It is noteworthy that the false-positive rate for MSS was much higher in ART-induced pregnancies (11.7%) than in SPs (3.2%). This might be explained by the difference in age (thus in risk level) between the two groups. We then restricted the analyses to the women with maternal age below 35 years. The difference between false-positive rates for MSS thus decreased in spontaneous and ART-induced pregnancies to 1.5% and 4.9%, respectively, with an intergroup difference of 3.4%. To explain this remaining gap, we took into account the maternal ages of 30.2 (27.9–32.7) for SP ($n = 339$) and 31.7 (29.4–33.4) for ART ($n = 165$) ($p < 1‰$), along with multiple of the median (MoM) for β-hCG respectively equal to 1.30 and 1.42 ($p = 0.17$) and MoM for PAPP-A respectively equal to 1.23 and 1.12 ($p = 0.06$). After adjustment for these variables, it was confirmed that the between-group difference in PAPP-A MoM was the main factor accounting for the higher rate of patients classified as high risk in the ART group (**Supplementary Table S1** online).

Additionally, the cfDNA assay performance was also evaluated for trisomy 18 and 13 screening, summarized in **Supplementary Table S2**. One patient was positive for trisomy 13 but did not choose to undergo invasive testing, as the fetus exhibited no abnormalities on ultrasound follow-up. Placental biopsies were performed at birth, with DNA examined by quantitative polymerase chain reaction showing abnormal profiles for markers located on chromosome 13, thus suggesting confined placenta mosaicism. The baby suffered from intrauterine growth restriction yet presented a normal karyotype at birth. No conclusion can be drawn for trisomy 18, given that there was no case in this study.

Table 3 Fetal fraction distribution in the spontaneous and ART pregnancy groups

Fetal fraction %	Spontaneous pregnancies	ART pregnancies
<10	39.4%	48.7%
10–15	50.4%	46.9%
≥15	10.1%	4.4%

ART, assisted reproduction technology.

Pregnancy outcomes

Pregnancy outcomes were assessed in the entire sample because the screening results were identical in spontaneous and ART-induced pregnancies. Among the 789 pregnant women analyzed, invasive procedures were performed in only 15 (1.9%), and in 7 of them intended to confirm the positive cfDNA assay results for T21, as currently recommended. All fetuses were confirmed as presenting T21. Eight additional fetuses were invasively examined due to ultrasound findings, and fetal conditions other than those screened for were thus revealed. The number of invasive tests was 10 in the SP group and 5 in the ART group, the difference being nonsignificant. Second-trimester scans were performed in 97% of cases, and third-trimester scans in 96% of cases. Of the 789 patients, 7 had T21 fetuses and 8 presented fetal anomalies that justified invasive sampling. Ultrasound follow-up was performed in 12 other patients for fetal anomalies yet without sampling (bowel duplication ($n = 1$), unique umbilical artery ($n = 1$), atrioventricular bloc ($n = 1$), intrauterine growth restriction ($n = 3$), pelvis dilatation <11 mm ($n = 4$), ovarian cyst ($n = 1$), and bilateral nephromegaly ($n = 1$)). All these neonates were examined by a pediatrician and assessed as phenotypically normal, with no karyotype testing. One fetus displayed left cardiac ventricular asymmetry on second-trimester scan, and the patient chose not to undergo invasive testing. At birth, the baby exhibited dysmorphic facial features, along with a cardiac malformation (double outlet ventricle). The parents refused karyotype analysis. Three patients exhibited intrauterine fetal death at 18, 22, and 39 weeks, with all karyotypes performed upon pathological examination and proven normal.

DISCUSSION

We herein report the first prospective, multicenter, interventional study evaluating the impact of introducing cfDNA as a first-line screening test in pregnant women, in the first trimester, with results of both MSS and cfDNA reported and considered for subsequent clinical management. Both SP and ART-induced pregnancies were considered because of conflicting results on the performance of cfDNA testing in ART-induced pregnancies, with MSS performance known to be impacted in that specific context.

We confirmed that cfDNA screening for T21 exhibits higher specificity and PPV than standard MSS, thus increasing the number of fetuses with T21 diagnosed while reducing the number of invasive procedures required by 78.5% even for a subgroup of low-risk patients under 35 years of age. Positive MSS findings were reported in 52 patients, while their cfDNA tests were negative, and none chose invasive testing. It must be noted that after receiving negative

MSS and cfDNA results, only eight patients underwent invasive testing. Of these eight patients with positive cfDNA results, four underwent amniocentesis rather than chorionic villus sampling. This was due partly to the delay in results turnaround.

When considering cfDNA as a first-tier screening, the "no-call" rate is a relevant metric, as patient management is often less than optimal in this context. The number of such cases was very low ($n=2$) in our study, with one relating to preanalytical issues. The patient did not accept a second blood sampling. Only one patient therefore had a "true no-call" result due to low FF, in spite of a normal BMI. This patient suffered from an autoimmune disease and was under heparin treatment. Whether this "no-call" result was related to either the heparin or the autoimmune disease is still unclear.[24]

Two recent meta-analyses confirmed the high performance of cfDNA testing, yet included both low-risk and high-risk patients,[3,25] which made it difficult to draw a definitive conclusion. To date, the two major publications evaluating cfDNA testing in the general population were published by Bianchi et al.[7] and Norton et al.[8] Only the second focused on the first trimester of pregnancy, however, while cfDNA results were blinded. Furthermore, information on the mode of conception was lacking, despite this being a relevant parameter when considering cfDNA as a primary screening tool, owing to the increasing number of ART-induced pregnancies worldwide. The performance of cfDNA testing in ART-induced pregnancies has already been evaluated in both twin[17,26,27] and singleton pregnancies.[28] For this reason, we compared the results of T21 screening between spontaneous and ART-induced pregnancy groups. The cfDNA testing performance was much higher than that of MSS in the SP group, with an even more striking difference in the ART group. This superiority in this latter group could be accounted for primarily by the mean maternal age, which was significantly higher in the ART group, but this difference persisted even when considering only patients under 35 years old. After adjusting for age, PAPP-A, and β-hCG MoM, however, we found lower PAPP-A MoM in the ART group to be responsible for the relatively poor performance of MSS found in this group. This applied at least to the false-positive results, which were responsible for the higher rate of invasive procedures.

Levels of the biomarkers used for first-trimester prenatal screening have been shown to be modified in ART-induced pregnancies as compared with SP. While decreased PAPP-A levels was the most common finding in several reports,[29–33] increased β-hCG levels have also been reported,[30] yet not in all studies.[29,33] It has been suggested that the type of ART procedure, such as IVF or ICSI, may influence marker levels,[30,32,33] with a direct impact on exogenous hormone levels, and thus ovarian stimulation. This modification is also controversial in the intrauterine insemination procedures.[33,34] However, its impact on the false-positive rate of first-trimester screening is debatable, as this must be adjusted for the ART procedure employed, given that different ART protocols and medical-history features seem to alter the screening parameters in different ways.[31,32,34–36] In our study, we showed that the performance of MSS is lower than that of cfDNA in ART-induced pregnancies, which may be due to differences in PAPP-A levels after adjusting for maternal age.

On the other hand, FF, a most relevant parameter, displayed a different distribution in SP and ART-induced pregnancies. Overall, though FF was lower in the ART group, both the mean level and number of cases under the critical 4% amount were similar in both groups. This finding might be related to PAPP-A lower level as both PAPP-A and cfDNA derived from syncytiotrophoblast.

Most countries are currently discussing how cfDNA testing should be optimally offered to patients from a medical, ethical, and economical point of view, as compared with MSS, which is still the current standard screening test. In France, where national screening has been implemented by law, first-trimester combined screening is still the standard care choice. Very recently, cfDNA testing was officially recognized as an option. However, on account of medico-economic evaluations,[37] this test is proposed only as a second-tier screening tool for high-risk patients after redefining the boundaries of such a group: the "new high-risk group" ranges from 1/51 to 1/1,000. Nonetheless, based on our findings, cfDNA testing should be considered as a primary screening method for trisomy 21, at least in patients with ART-induced pregnancies with the objective to reduce invasive procedures or for those with sole access to second-trimester biochemical screening where the rate of high-risk patients is especially high in our study (data not shown). Moreover, based on our findings, cfDNA testing should also be used for trisomy 13 and 18 screening, and this especially in countries where such risk assessment is not covered by the MSS, as it is in France.

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/gim

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DISCLOSURE

J.M.C., L.L., and P.K. are employees and shareholders of CERBA. The other authors declare no conflict of interest.

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