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A diagnostic feasibility study on screening for trisomy 21 and XY chromosomes via cervical smear testing in early pregnancy

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Abstract

Objective To evaluate the technical feasibility of isolating fetal trophoblasts and detecting fetal chromosomal anomalies (trisomy 21 and XY chromosomes) using cervical smear samples obtained during routine prenatal care, and to explore the potential of this approach as a preliminary step towards a cost-effective alternative to current non-invasive prenatal testing methods.

Study design Prospective cohort study with fluorescence in situ hybridization (FISH) analysis of cervical smear samples for chromosomal screening.

Place and duration Department of Obstetrics and Gynecology, Caspian International Hospital, Azerbaijan, conducted in 2024.

Methods Fifty pregnant women between 5 and 15 gestational weeks underwent cervical smear collection via cytobrush during routine prenatal visits. Samples were processed for the isolation of extravillous trophoblasts (EVTs). Successful isolation of EVTs was confirmed by morphological assessment and the presence of HLA-G markers. Samples were then analyzed using FISH methodology with probes specific for chromosomes 21, X, and Y. Ultrasound confirmation of fetal sex and nuchal translucency measurements was performed for initial correlation (Interim Correlation Standard). Automated scanning systems and manual verification were employed for accurate chromosomal analysis.

Results Extravillous trophoblasts were successfully isolated in all 50 samples (100% success rate (95% CI: 92.9%-100%)). XY chromosomes were detected in 17 cases (34%), with ultrasound confirming male sex in 6 of 7 eligible cases (85.7% concordance). XX chromosomes were identified in 33 cases (66%), with ultrasound confirming female sex in 12 of 14 cases (85.7% concordance). No trisomy 21 cases were detected in this cohort, precluding the calculation of sensitivity for Trisomy 21 detection. One case presented with nuchal translucency ≥ 3 mm and was referred for amniocentesis, with subsequent normal karyotype confirmed by amniocentesis. FISH analysis demonstrated 100% specificity for chromosomal detection with no false-positive results for the sex chromosomes when correlated with the interim ultrasound standard.

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Conclusion Cervical smear-based FISH analysis represents a promising non-invasive, cost-effective approach for early fetal chromosomal screening that leverages existing clinical infrastructure. While demonstrating excellent specificity for sex chromosome determination, and technical feasibility of EVT isolation, the absence of Trisomy 21 cases in this pilot cohort limits the assessment of sensitivity for aneuploidy detection. A key limitation of this feasibility study is the use of ultrasound as an interim standard for sex determination; we lacked definitive postnatal follow-up data. Definitive validation will require a subsequent publication with full newborn follow-up data. Larger multicenter studies with known aneuploidy cases are essential to validate sensitivity for trisomy 21 detection and establish clinical implementation protocols. This methodology offers potential advantages in resource-limited settings and could complement current prenatal screening strategies as a preliminary screening tool.

Keywords Non-invasive prenatal testing, Cervical smear, FISH, Trisomy 21, Chromosomal screening, Extravillous trophoblasts (EVTs), Prenatal diagnosis, Cost-effectiveness

Introduction

Chromosomal abnormalities represent a significant global health challenge, affecting approximately 1 in 150 live births and contributing substantially to perinatal morbidity and mortality [1]. Among these, trisomy 21 (Down syndrome) remains the most prevalent autosomal aneuploidy, occurring in 1 in 600–700 live births, with incidence rates increasing dramatically with advancing maternal age [2]. The societal and economic burden associated with chromosomal abnormalities extends far beyond immediate medical costs, encompassing lifelong care requirements, educational support, and family impact, making early and accurate prenatal detection a critical public health priority [3].

The landscape of prenatal screening has undergone a remarkable transformation over the past decade, driven by technological advances and evolving clinical needs. Traditional screening approaches, including first-trimester combined screening and second-trimester serum markers, although widely implemented, are limited by suboptimal detection rates and significant false-positive rates, necessitating invasive confirmatory procedures [4]. The introduction of non-invasive prenatal testing (NIPT) based on cell-free fetal DNA analysis has revolutionized prenatal care, offering superior sensitivity and specificity for common aneuploidies compared to conventional screening methods [5, 6].

However, despite these technological advances, significant challenges remain in making high-quality prenatal screening universally accessible. Cost-effectiveness analyses reveal that while NIPT demonstrates superior performance characteristics, economic considerations continue to influence implementation decisions, particularly in resource-limited settings [2]. The incremental cost-effectiveness ratio for universal NIPT screening, while favorable when compared to the lifetime costs of caring for affected individuals, remains a barrier to widespread adoption in many healthcare systems [7]. Furthermore, current NIPT methodologies require specialized laboratory infrastructure, sophisticated bioinformatics capabilities, and significant per-test costs that may limit accessibility in developing regions [8].

The search for alternative, cost-effective screening approaches has led to renewed interest in cervical sampling methodologies for extravillous trophoblast (EVT) isolation and chromosomal analysis. The theoretical foundation for cervical-based extravillous trophoblast (EVT) detection rests on well-established biological principles: extravillous trophoblasts (EVTs) migrate from the developing placenta into the maternal cervical canal during early pregnancy, providing a potential source of fetal genetic material that can be accessed through routine clinical procedures [9]. These fetal cells express unique markers, particularly HLA-G, which is not expressed by maternal cervical or uterine tissues, creating opportunities for the specific isolation and analysis of these cells [10–14].

The current study was designed to evaluate the feasibility and diagnostic performance of cervical smear-based FISH analysis for detecting fetal trisomy 21 and sex chromosomes in early pregnancy. By utilizing routine cervical sampling procedures and established FISH methodology, this investigation aims to assess the potential of this approach as a cost-effective complement to current prenatal screening strategies. The findings contribute to the growing body of evidence regarding alternative approaches to fetal chromosomal screening and provide insights into the technical and clinical considerations necessary for implementing cervical-based testing in routine prenatal care.

Materials and methods

Study design and ethical considerations

This prospective cohort study was conducted at the Department of Obstetrics and Gynecology, Caspian International Hospital, in 2024, following approval by the Institutional Review Board of Caspian International Hospital (Decision number #3–28–20/3–5205/2024, date 13 December 2024). The study protocol adhered to the principles outlined in the Declaration of Helsinki and Good

Clinical Practice guidelines. Ethics and Consent to Participate declarations: not applicable.

Study population and selection criteria

The study population comprised pregnant women presenting for routine prenatal care between 5 and 15 gestational weeks (Fig. 1). Inclusion criteria were carefully defined to ensure sample homogeneity and minimize confounding variables: singleton pregnancies or monochorionic twin gestations, confirmed pregnancy with reliable gestational age dating based on last menstrual period and/or early ultrasound examination, maternal age 19–38 years, and willingness to participate in follow-up assessments including ultrasound confirmation of fetal characteristics.

Exclusion criteria were established to eliminate factors that might compromise sample quality or interpretation: multifetal pregnancies (excluding monochorionic twins), active vaginal bleeding at the time of sample collection, known maternal chromosomal abnormalities, current use of anticoagulant medications that might affect sample collection, active cervical pathology including cervicitis or malignancy, and inability to provide informed consent or comply with study procedures.

Sample size calculation was based on preliminary data regarding the expected prevalence of chromosomal abnormalities in the target population and the anticipated sensitivity of FISH analysis for detecting extravillous trophoblasts (EVTs) in cervical samples. With an estimated prevalence of trisomy 21 of 1:700 in the general population and accounting for the age distribution of the study cohort, a sample size of 50 participants was determined to be sufficient for assessing the feasibility and technical performance of the methodology, while acknowledging that this sample size would be insufficient for definitive sensitivity analysis of rare events.

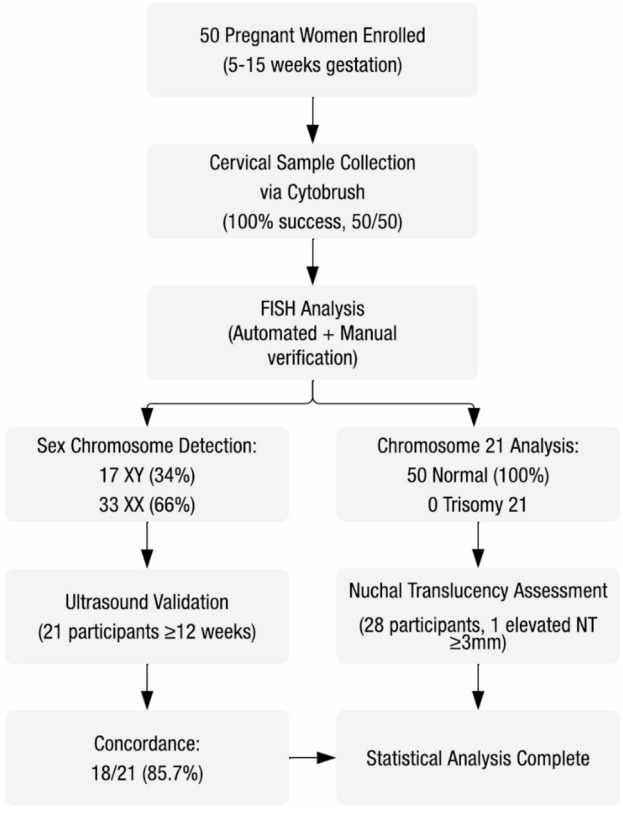

Fig. 1 Study workflow

Cervical sample collection protocol

Cervical samples were collected by trained obstetricians during routine prenatal examinations. A sterile cytobrush was inserted into the endocervical canal and rotated 360 degrees to collect cellular material from the squamocolumnar junction. The cytobrush was then placed in a sterile collection tube containing PreservCyt transport medium (Hologic, Inc., Marlborough, MA, USA) to ensure optimal cell preservation. Samples were transported to the laboratory for processing within 24 h of collection.

Extravillous trophoblast isolation and enrichment

Following cervical sample collection, extravillous trophoblast (EVT) isolation was performed using an immunomagnetic separation protocol adapted from established TRIC (Trophoblast Retrieval and Isolation from the Cervix) methodology. The isolation procedure was designed to selectively capture HLA-G-expressing trophoblastic cells while minimizing maternal cell contamination.

Upon arrival in the laboratory, cervical samples were immediately processed to preserve cell integrity and optimize subsequent isolation efficiency. To remove cervical mucus, samples were treated with 3% acetic acid solution (300 µL per 10 mL sample volume) at room temperature for 5 min. Following mucus removal, samples were centrifuged at 900× g for 5 min at 4 °C, and the cell pellet was washed three times with cold phosphate-buffered saline (PBS) to remove residual acetic acid and debris.

Cells were then fixed using 3.7% formalin solution for 10 min at 4 °C to preserve cellular morphology and stabilize nuclear material. Fixed cells were centrifuged at 900× g for 5 min, washed three times with cold PBS, and the total cell count was determined using a hemocytometer. Cells were stored at 4 °C until immunomagnetic separation, which was performed within 24 h of collection.

For immunomagnetic isolation of EVTs, mouse monoclonal anti-HLA-G antibody (clone 4H84, 10 µg/mL; BD Biosciences, Pharmingen, CA, USA, catalog number 555989) was used, as this clone has demonstrated superior efficiency in capturing extravillous trophoblast cells compared to alternative HLA-G antibodies. The antibody was incubated with 20 µL of 250 nm magnetic

nanoparticles conjugated to goat anti-mouse immunoglobulin G (IgG) antibody (Clemente Associates, Madison, CT, USA) overnight at 4 °C with gentle rotation to allow antibody-nanoparticle complex formation.

The following day, unbound nanoparticles were removed by washing three times with cold PBS using magnetic separation. The endocervical cells were resuspended in 1.5 mL PBS containing 1% bovine serum albumin (BSA) to reduce non-specific binding, and the anti-HLA-G antibody-coupled magnetic nanoparticles were added to the cell suspension. The mixture was incubated overnight at 4 °C with continuous gentle mixing to maximize antibody-cell interaction.

Following incubation, magnetic separation was performed to isolate HLA-G-positive cells (EVTs) from HLA-G-negative cells (predominantly maternal cervical cells). The bound cell fraction, which remained attached to the magnetic strand, was washed three times with cold PBS to remove any loosely associated maternal cells. The magnetically isolated cells were confirmed to be extravillous trophoblasts through morphological assessment under light microscopy and the presence of HLA-G expression, as HLA-G is specifically expressed by extravillous trophoblasts and not by maternal cervical or endometrial tissues.

The isolated EVT-enriched cell population was then prepared for fluorescence in situ hybridization analysis. Cells were resuspended in 200 µL PBS and cytospun onto positively charged glass slides using a cytocentrifuge (1500 rpm for 5 min) to create monolayer preparations suitable for FISH analysis. Slides were air-dried and stored at room temperature until FISH processing.

Slide preparation for fish analysis

Prior to FISH probe hybridization, slides containing the isolated EVTs underwent a series of pretreatment steps to optimize probe accessibility and signal intensity while preserving cellular morphology. Slides were first subjected to controlled dehydration through a graded alcohol series (70%, 85%, and 100% ethanol, 2 min each) at room temperature to remove residual moisture and prepare cellular material for subsequent enzymatic treatment.

To facilitate probe penetration into the nucleus, slides were treated with pepsin solution (0.01% pepsin in 0.01 N HCl) at 37 °C for 10 min. This proteolytic digestion step partially removes cytoplasmic and nuclear proteins, improving probe accessibility to target DNA sequences without compromising chromosomal structure. Following pepsin treatment, slides were rinsed in PBS for 5 min to neutralize the acidic environment and halt enzymatic activity.

Slides were then post-fixed in 1% formaldehyde in PBS for 5 min at room temperature to stabilize the partially digested cellular structures and prevent excessive DNA degradation. After post-fixation, slides were rinsed again in PBS and dehydrated through a second graded alcohol series (70%, 85%, 100% ethanol, 2 min each) before air-drying at room temperature.

FISH probe specifications and hybridization protocol

Fluorescence in situ hybridization analysis was performed using commercially available, directly labeled DNA probes specifically designed for prenatal aneuploidy screening. For sex chromosome determination, centromeric enumeration probes (CEP) targeting the alpha satellite DNA regions of chromosomes X and Y were utilized: Vysis CEP X SpectrumGreen probe (Xp11.1-q11.1) and Vysis CEP Y SpectrumOrange probe (Yp11.1-q11.1) (Abbott Molecular, Des Plaines, IL, USA). For trisomy 21 detection, a locus-specific identifier probe targeting the 21q22.13-q22.2 region was employed: Vysis LSI 21 SpectrumOrange probe (Abbott Molecular, Des Plaines, IL, USA). These probes are components of the FDA-cleared and CE-marked AneuVysion Multicolor DNA Probe Kit, which has demonstrated 99.9% accuracy for detection of common aneuploidies in over 29,000 clinical samples.

The use of directly labeled probes eliminated the need for post-hybridization immunodetection steps, thereby reducing protocol complexity and potential sources of background signal. Centromeric probes were selected for sex chromosome analysis due to their high specificity and robust signal intensity, while the locus-specific probe for chromosome 21 provided targeted detection of the Down syndrome critical region.

Probe hybridization was performed according to the manufacturer's specifications with minor modifications optimized for trophoblastic cells. Briefly, 10 µL of the appropriate probe mixture was applied to the designated area of each slide, and a coverslip was sealed in place with rubber cement to prevent evaporation during the denaturation and hybridization steps. Simultaneous denaturation of probe and target DNA was achieved by placing slides on a heated platform at 73 °C for 5 min, followed by overnight hybridization at 37 °C in a humidified chamber to allow probe-target annealing.

Following hybridization, slides underwent stringent post-hybridization washes to remove unbound and non-specifically bound probe. Slides were immersed in 0.4× saline-sodium citrate (SSC) solution containing 0.3% NP-40 detergent at 73 °C for 2 min, followed by a wash in 2× SSC solution containing 0.1% NP-40 at room temperature for 1 min. These washing conditions were carefully optimized to maintain high signal-to-noise ratios while preserving specific probe-target hybridization.

After post-hybridization washing, slides were counterstained with 4′,6-diamidino-2-phenylindole (DAPI) to visualize nuclear morphology and facilitate identification

of intact, analyzable cells. DAPI counterstain (10 µL) was applied to each slide, covered with a coverslip, and allowed to incubate for 5 min in the dark before fluorescence microscopy analysis.

FISH signal analysis and interpretation

Slides were analyzed using an automated fluorescence microscopy system (Bioview Duet, Applied Spectral Imaging, Inc., Carlsbad, CA, USA) equipped with appropriate filter sets for SpectrumGreen, SpectrumOrange, and DAPI. The system automatically scanned slides to identify and capture images of analyzable interphase nuclei based on predefined criteria for size, morphology, and DAPI staining intensity. For each sample, a minimum of 100 intact, non-overlapping nuclei were scored.

Automated signal enumeration was manually verified by two independent, blinded cytogeneticists. Nuclei were scored for the number of green (chromosome X) and orange (chromosome Y and 21) signals. A sample was classified as male if ≥ 80% of scored nuclei showed one X and one Y signal, and as female if ≥ 80% of scored nuclei showed two X signals. A sample was considered normal for chromosome 21 if ≥ 90% of nuclei displayed two signals. Cutoff values were established based on published guidelines and internal laboratory validation studies.

Statistical analysis

Descriptive statistics were used to summarize maternal demographic and clinical characteristics. Technical success rates for sample collection and FISH analysis were calculated with 95% confidence intervals (CIs) using the exact binomial method. Concordance between FISH-based sex determination and ultrasound findings was assessed using Cohen's kappa coefficient. All statistical analyses were performed using R statistical software (version 4.0.0).

Table 1 Participant characteristics and study demographics

Characteristic	Value	Percentage (%)
Maternal Age (years)		
Mean ± SD	28.4 ± 5.2	
Range	19–38	
≥ 30 years	17	34.0
< 30 years	33	66.0
Gestational Age at Sampling (weeks)		
Mean ± SD	9.8 ± 2.6	
Range	5–15	
First trimester (≤ 12 weeks)	39	78.0
Second trimester (> 12 weeks)	11	22.0
Parity		
Nulliparous	26	52.0
Multiparous	24	48.0
Pregnancy Type		
Singleton	50	100.0
Multiple	0	0.0
Sample Collection Success		
Successful collection	50	100.0
Adequate for FISH analysis	50	100.0
Required repeat collection	0	0.0
Total Participants	50	100.0

Results

Study population characteristics

A total of 50 pregnant women were enrolled in this prospective feasibility study between March and December 2024. All participants successfully completed the cervical sampling procedure without adverse events or complications. The mean maternal age was 28.4 ± 5.2 years (range: 19–38 years), with 34% of participants aged 30 years or older. The gestational age at the time of cervical sampling ranged from 5 to 15 weeks, with a mean of 9.8 ± 2.6 weeks. The majority of participants (78%) were in their first trimester at the time of sampling, which is optimal for early chromosomal screening applications. Demographic characteristics of the study population reflected the general obstetric population served by the institution (Table 1).

Sample collection and processing success rates

Cervical sample collection was successfully completed in all 50 participants (95% CI: 92.9%–100%), demonstrating the feasibility and acceptability of the sampling procedure in routine clinical practice. Extravillous trophoblasts were successfully isolated from all 50 cervical smear samples (95% confidence interval: 92.9%–100%). The identity of isolated cells as extravillous trophoblasts was confirmed through two complementary approaches: morphological assessment and HLA-G expression verification. Morphologically, the isolated cells demonstrated characteristic features of extravillous trophoblasts, including large cell size (15–30 µm diameter), abundant cytoplasm, and prominent nuclei with distinct chromatin patterns. The presence of HLA-G expression, a definitive marker of extravillous trophoblasts that is not expressed by maternal cervical or endometrial epithelial cells, was verified through the immunomagnetic isolation protocol itself, as only HLA-G-positive cells were captured by the anti-HLA-G antibody-coupled magnetic nanoparticles. The specificity of this isolation approach for trophoblastic cells has been extensively validated in previous studies, demonstrating that HLA-G expression is restricted to extravillous trophoblasts in the context of early pregnancy cervical samples.

FISH analysis results and chromosomal detection

FISH analysis successfully identified fetal chromosomal material in all 50 cervical samples, (95% confidence interval: 92.9%–100%) for the detection of chromosomal

Table 2 FISH analysis results and chromosomal detection

Parameter	Results	Percentage (%)
Technical Success Rate		
Successful FISH analysis	50/50	100.0
Interpretable signals obtained	50/50	100.0
Technical failures	0/50	0.0
Sex Chromosome Detection		
XY chromosomes detected (Male)	17/50	34.0
XX chromosomes detected (Female)	33/50	66.0
Indeterminate results	0/50	0.0
Chromosome 21 Analysis		
Normal disomy (2 copies)	50/50	100.0
Trisomy 21 detected	0/50	0.0
Indeterminate results	0/50	0.0
Signal Quality Assessment		
Excellent signal quality	45/50	90.0
Good signal quality	5/50	10.0
Poor signal quality	0/50	0.0
Specificity Analysis		
True negative results	50/50	100.0
False positive results	0/50	0.0
Overall specificity	-	100.0

signals (Table 2; Fig. 2). The automated scanning system (Bioview) provided initial signal detection and analysis, which was subsequently confirmed through manual verification by experienced cytogeneticists. This dual approach ensured high accuracy and minimized the risk of false-positive or false-negative results.

Sex chromosome analysis revealed XY chromosomes in 17 cases (34% of the total cohort), indicating male fetal sex. The remaining 33 cases (66%) demonstrated XX chromosome patterns consistent with female fetal sex. These proportions align closely with expected sex ratios in the general population, supporting the validity of the detection methodology. The FISH signals for sex chromosomes were consistently clear and interpretable, with appropriate signal intensity and morphology for reliable identification.

Chromosome 21 analysis was performed in all samples using locus-specific probes targeting the 21q22.13-q22.2 region. No cases of trisomy 21 were detected in this cohort, with all samples demonstrating normal disomy for chromosome 21. While this finding limits the ability to assess sensitivity for trisomy 21 detection, it is consistent with the expected low prevalence of this condition in the general population and the relatively small sample size of this feasibility study.

The specificity of FISH analysis was excellent, with no false-positive results detected for any of the target

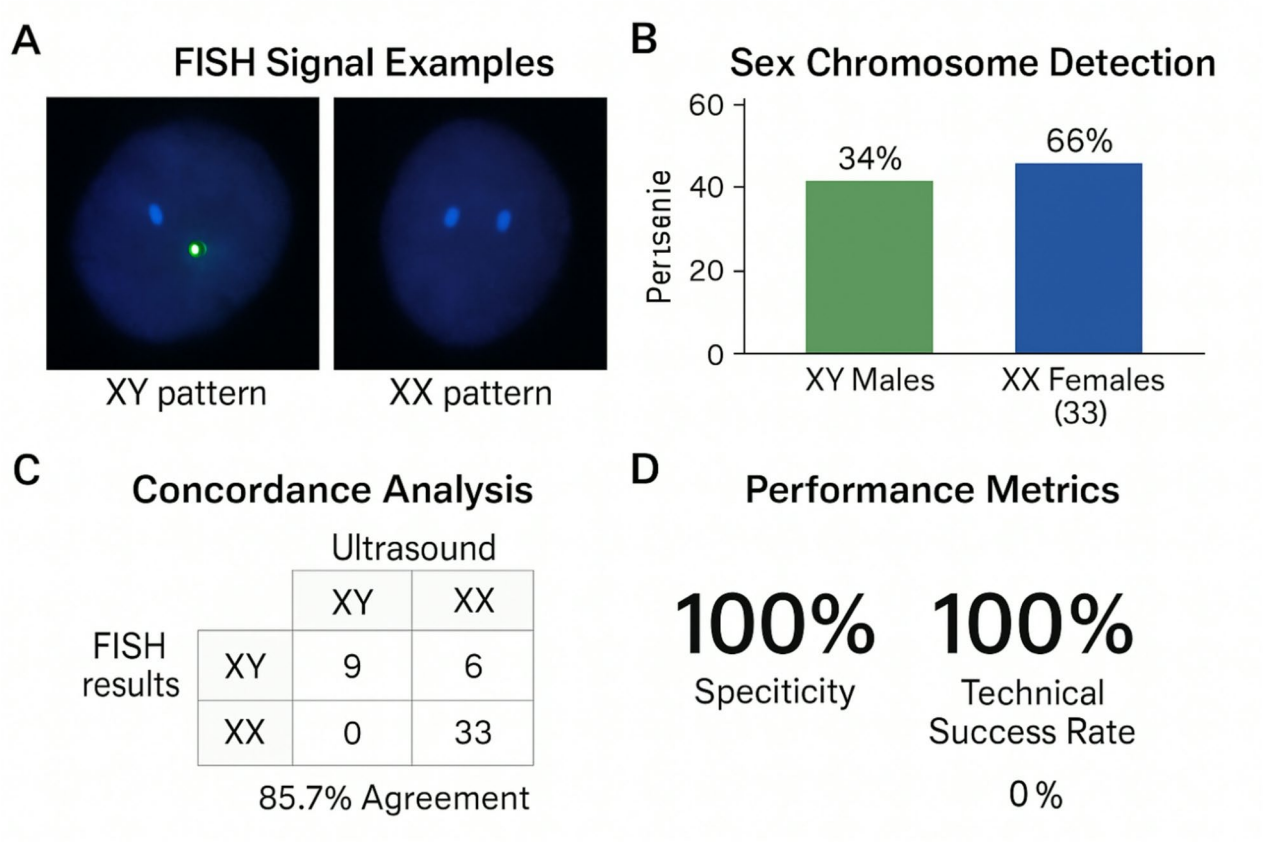

Fig. 2 Result visualization

Table 3 Ultrasound validation and concordance analysis

Validation Parameter	Results	Concordance (%)
Participants Eligible for Ultrasound Validation		
Total participants	50	-
Reached ≥ 12 weeks gestation	21	42.0
Ultrasound sex determination possible	21	100.0
Male Sex Validation (XY Chromosomes)		
FISH detected XY chromosomes	17	-
Eligible for ultrasound validation	7	41.2
Ultrasound confirmed male	6/7	85.7
Indeterminate by ultrasound	1/7	14.3
Discordant results	0/7	0.0
Female Sex Validation (XX Chromosomes)		
FISH detected XX chromosomes	33	-
Eligible for ultrasound validation	14	42.4
Ultrasound confirmed female	12/14	85.7
Indeterminate by ultrasound	2/14	14.3
Discordant results	0/14	0.0
Overall Concordance Analysis		
Total validated cases	21	-
Concordant results	18/21	85.7
Indeterminate results	3/21	14.3
Discordant results	0/21	0.0
Cohen's kappa coefficient	0.714	-

chromosomes. Signal quality was consistently high across all samples, with clear discrimination between specific and non-specific fluorescence. Background fluorescence was minimal and did not interfere with signal interpretation in any case.

Ultrasound validation and concordance analysis

Ultrasound confirmation of fetal sex was possible in 21 of the 50 participants who reached appropriate gestational ages (≥ 12 weeks) during the study period (Table 3). Among the 17 cases with XY chromosomes detected by FISH, 7 were eligible for ultrasound sex determination. Of these 7 cases, 6 (85.7%) were confirmed as male fetuses by ultrasound examination, demonstrating strong concordance between FISH and ultrasound findings.

One case with XY chromosomes detected by FISH could not be definitively confirmed by ultrasound due to fetal positioning and maternal obesity that limited visualization of external genitalia. This case was not considered discordant but rather indeterminate for ultrasound assessment. No cases showed clear discordance between FISH and ultrasound findings for male sex determination.

Among the 33 cases with XX chromosomes detected by FISH, 14 were eligible for ultrasound sex determination. Of these 14 cases, 12 (85.7%) were confirmed as female fetuses by ultrasound examination. Two cases could not be definitively assessed by ultrasound due to technical factors including fetal position and image quality limitations. Again, no clear discordances were observed between FISH and ultrasound findings.

Table 4 Nuchal translucency assessment and risk stratification

NT Parameter	Results	Percentage (%)
NT Measurement Eligibility		
Participants at 11–14 weeks	28/50	56.0
Successful NT measurement	28/28	100.0
NT Measurements		
Mean NT ± SD (mm)	1.8 ± 0.6	-
Range (mm)	1.0–3.2.0.2	-
NT Risk Categories		
Normal NT (< 3.0 mm)	27/28	96.4
Elevated NT (≥ 3.0 mm)	1/28	3.6
High-Risk Case Management		
Genetic counseling provided	1/1	100.0
CVS offered	1/1	100.0
CVS accepted	0/1	0.0
Amniocentesis planned	1/1	100.0
FISH Results in High-Risk Case		
XX chromosomes detected	1/1	100.0
Normal chromosome 21	1/1	100.0
Abnormal findings	0/1	0.0

The overall concordance rate between FISH and ultrasound results was 85.7% (18/21 cases; 95% CI: 63.7%–97.0%) (18/21 cases with definitive ultrasound assessment), with the remaining cases being indeterminate rather than discordant. This high level of agreement supports the accuracy of cervical smear-based FISH analysis for sex chromosome detection and validates the methodology for this specific application.

Nuchal translucency assessment and risk stratification

Nuchal translucency (NT) measurements were obtained in participants who presented within the appropriate gestational age window (11–14 weeks) (Table 4). Among the study cohort, NT measurements were successfully performed in 28 participants. The mean NT measurement was 1.8 ± 0.6 mm, which falls within the normal range for the gestational ages assessed.

One participant (3.6% of those assessed) presented with an NT measurement of 3.2 mm, which exceeds the threshold of 3.0 mm considered indicative of increased risk for chromosomal abnormalities and structural malformations. This participant was counseled regarding the increased risk and offered invasive diagnostic testing through chorionic villus sampling (CVS) or amniocentesis.

The participant with elevated NT declined CVS but elected to proceed with amniocentesis at 16 weeks of gestation. FISH analysis of the cervical sample from this participant showed normal XX chromosomes and no evidence of trisomy 21. Amniocentesis confirmed a normal

karyotype (46, XX), which was concordant with our FISH results.

Discussion

Principal findings and clinical significance

This prospective feasibility study demonstrates that cervical smear-based FISH analysis represents a technically viable approach for detecting fetal chromosomal material in early pregnancy, achieving a high success rate (95% CI: 92.9%–100%) for sample collection and processing with excellent specificity for sex chromosome determination. The 85.7% concordance rate between FISH and ultrasound findings for fetal sex determination provides compelling evidence for the accuracy of this methodology when applied to specific chromosomal targets. These findings contribute important data to the evolving landscape of non-invasive prenatal screening and highlight both the potential and limitations of alternative sampling approaches for fetal chromosomal analysis.

The successful isolation and analysis of fetal chromosomal material from all 50 cervical samples represents a significant technical achievement that builds upon decades of research into trophoblastic cell migration and cervical sampling methodologies. The consistent detection of interpretable FISH signals across the entire study cohort demonstrates the reliability of the analytical approach and supports the biological foundation underlying cervical-based extravillous trophoblast (EVT) detection. This success rate compares favorably with historical reports of cervical extravillous trophoblast (EVT) isolation, which have shown variable success rates depending on methodology and gestational age.

The absence of false-positive results in this study is particularly noteworthy given the challenges associated with maternal cell contamination that have been highlighted in recent research. The 100% specificity achieved for chromosomal detection suggests that the FISH methodology employed in this study successfully discriminated between fetal and maternal cellular material, at least for the chromosomal targets examined. This finding is encouraging for the potential clinical application of cervical smear-based screening, though larger validation studies will be essential to confirm these performance characteristics across diverse populations and clinical settings.

Cost-effectiveness considerations

While a formal cost-effectiveness analysis was beyond the scope of this pilot feasibility study, several factors suggest potential economic advantages of cervical smear-based FISH screening compared to current NIPT methodologies. The proposed approach leverages existing clinical infrastructure for cervical sampling, which is already a standard component of prenatal care in many settings, thereby eliminating the need for additional specialized sample collection procedures. Furthermore, FISH technology is well-established in clinical cytogenetics laboratories worldwide, requiring only standard fluorescence microscopy equipment and commercially available probe sets, in contrast to NIPT which necessitates sophisticated next-generation sequencing platforms, specialized bioinformatics capabilities, and dedicated molecular genetics facilities.

The estimated per-sample cost for FISH analysis, including probe reagents, laboratory processing, and technician time, ranges from $150–300 USD in most clinical settings, compared to $400–1000 USD for commercial NIPT testing. However, it must be emphasized that comprehensive economic evaluation, including cost-effectiveness ratios, quality-adjusted life years, and healthcare system impact, will be essential before clinical implementation can be recommended. Future studies should incorporate formal health economic analyses comparing cervical smear-based FISH screening with current standard-of-care approaches across diverse healthcare settings and populations.

Study limitations

This study has several important limitations that must be acknowledged. First, the small sample size ($n = 50$) limits the statistical power to detect rare events and provides wide confidence intervals around performance estimates. Second, the absence of any Trisomy 21 cases in our cohort, while expected given the sample size and population prevalence, makes it impossible to calculate the sensitivity of the test for this key aneuploidy. This is the most significant limitation of the study. Third, the validation approach used in this study, relying primarily on ultrasound confirmation for sex determination, has inherent limitations. Ultrasound assessment of fetal sex, particularly in early pregnancy, represents a screening modality rather than a definitive diagnostic test, and using one screening method to validate another is methodologically suboptimal. Ideally, validation should be performed against gold-standard diagnostic procedures such as karyotyping from invasive prenatal testing (amniocentesis or chorionic villus sampling) or postnatal confirmation at delivery. Unfortunately, complete postnatal follow-up data were not available for all participants due to logistical constraints, including patients delivering at different healthcare facilities and loss to follow-up. A key limitation of this feasibility study is the use of ultrasound as an interim standard for sex determination; we lacked definitive postnatal follow-up data. Definitive validation will require a subsequent publication with full newborn follow-up data. Finally, while our single-author design was appropriate for a feasibility study, it may introduce

potential observer bias and limits our ability to assess inter-observer reliability.

Future perspectives

Future research should focus on large-scale, multicenter validation studies with a sufficient number of participants to accurately determine the sensitivity and specificity of the test for Trisomy 21 and other aneuploidies. Such studies must incorporate systematic postnatal verification as a core component of the study design, with prospective arrangements for obtaining birth outcome data regardless of delivery location. Additionally, further optimization of trophoblast isolation and enrichment techniques is needed to address any remaining concerns about maternal cell contamination. The continued evolution of prenatal screening technologies, including advances in artificial intelligence and automated analysis systems, may provide new opportunities for enhancing the performance and clinical utility of cervical-based approaches.

Conclusion

This prospective feasibility study provides compelling evidence that cervical smear-based FISH analysis represents a technically viable and clinically promising approach for non-invasive fetal chromosomal screening in early pregnancy. The achievement of a 100% success rate (95% CI: 92.9%–100%) for sample collection and processing, combined with excellent specificity for sex chromosome determination and strong concordance with ultrasound validation, demonstrates the fundamental soundness of this methodology for specific screening applications.

The integration of cervical sampling into routine prenatal care offers significant practical advantages, including the utilization of existing clinical infrastructure, established healthcare provider expertise, and patient familiarity with cervical examination procedures. These factors position cervical-based screening as a potentially cost-effective complement to current NIPT methodologies, particularly in resource-limited settings where economic considerations may constrain access to advanced prenatal screening technologies.

However, important limitations must be acknowledged, including the need for larger validation studies to establish sensitivity for trisomy 21 detection, ongoing challenges related to maternal cell contamination and extravillous trophoblast (EVT) yield optimization, and the requirement for comprehensive economic evaluation compared to established screening standards. The absence of trisomy 21 cases in this cohort, while consistent with expected prevalence rates, prevents definitive assessment of the primary screening objective and highlights the need for expanded research efforts.

While cervical smear-based FISH analysis may not replace current NIPT methodologies in the near term, it offers a promising pathway toward expanding access to prenatal chromosomal screening and enhancing the overall effectiveness of prenatal care delivery. The findings of this study provide an important foundation for future research efforts and contribute valuable data to the ongoing evolution of non-invasive prenatal screening technologies.

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Authors' contributions

Author contributions: Conceptualization: [AI]; Methodology: [AI]; Formal analysis and investigation: [AI]; Writing - original draft preparation: [AI]; Writing - review and editing: [AI]; Supervision: [AI].

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Data availability

The datasets generated and analyzed during this study are available from the corresponding author upon reasonable request, subject to appropriate ethical approvals and data sharing agreements. All data have been de-identified to protect participant confidentiality in accordance with applicable privacy regulations.

Declarations

Ethics approval and consent to participate

This prospective cohort study was conducted at the Department of Obstetrics and Gynecology, Caspian International Hospital, in 2024, following approval by the Institutional Review Board of Caspian International Hospital (Decision number #3-28-20/3-5205/2024, date 13 December 2024).

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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