Received: 13 February 2025 | Accepted: 17 April 2025

DOI: 10.1002/rmb2.12655

Reproductive Medicine and Biology

WILEY

ORIGINAL ARTICLE

# Comparative prospective study on the clinical utility of G-banding and next-generation sequencing for chromosomal analysis of products of conception under Advanced Medical Care A in Japan

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## Abstract

**Purpose:** To compare the clinical utility of G-banding and next-generation sequencing (NGS) for chromosomal analysis of products of conception (POC), a crucial tool for detecting fetal chromosomal abnormalities which are major causes of miscarriage and stillbirth.

**Methods:** We evaluated the clinical utility of both techniques in a prospective analysis of 40 patients who experienced miscarriages or stillbirths between 6 and 36 weeks of gestation under Advanced Medical Care A in Japan. Both methods were applied to the same POC samples. The primary outcome was the proportion of patients with a presumed cause of miscarriage or stillbirth among all submitted samples.

**Results:** NGS presumed the cause in 75.0% (30/40) of cases, significantly outperforming G-banding's 42.5% (17/40) ($p < 0.01$). G-banding could analyze 67.5% (27/40) of the samples owing to culture failure, whereas NGS successfully analyzed all samples (100%, 40/40) ($p < 0.01$). Among the successfully analyzed samples, NGS presumed the cause in 70.3% (19/27) of cases, compared with 62.9% (17/27) for G-banding ($p = 0.31$). For miscarriages before 12 weeks, NGS presumed the cause in 73.5% (25/34) of cases, significantly higher than the 44.1% (15/34) ($p < 0.01$) presumed using G-banding.

**Conclusions:** These results highlight the superior efficacy of NGS over G-banding for presuming causes of miscarriage or stillbirth.

KEYWORDS
fetal chromosomal abnormalities, G-banding, miscarriage, next-generation sequencing, products of conception

Hidemine Honda and Tsuyoshi Takiuchi contributed equally to this work.

## 1 | INTRODUCTION

The frequency of miscarriages and stillbirths is significant, affecting ~30% of clinical pregnancies, which varies according to maternal age.[1] The causes of miscarriage and stillbirth can be categorized into fetal and maternal factors, with fetal factors accounting for the majority.[2] The rate of miscarriages increases with advancing maternal age, exceeding 50% in pregnancies among women aged 43 and older.[1] This increase is partially attributed to the age-related increase in aneuploid oocytes, beginning in the late 20s.[3] Consequently, the prevalence of chromosomal abnormalities in embryos increases with maternal age. Fetal chromosomal abnormalities are the leading cause of miscarriage.[4–6] Maternal factors, including uterine malformations, endocrine disorders, immune diseases, and thrombotic disorders, also contribute significantly to miscarriages and stillbirths.[7] Regardless of the underlying cause (whether fetal or maternal factors), identifying the reasons and risk factors is essential for stabilizing maternal mental health and guiding future treatment strategies. Various guidelines recommend conducting products of conception (POC) analysis after a second miscarriage as it aids in determining future treatment strategies.[8,9]

Traditionally, G-banding, requiring cell culture, is used for the morphological analysis of chromosome numbers and structures.[10] However, this method presents several limitations. First, following the natural expulsion of POC, bacterial contamination compromises the cell culture, thereby making the analysis infeasible and typically necessitating dilation and evacuation (D&E).[11] Additionally, G-banding requires fresh samples; however, patients diagnosed with miscarriages are often too emotionally distressed to decide on immediate chromosomal testing, hence preventing the freezing of POC samples for subsequent testing. Furthermore, G-banding occasionally yields inconsistent and unstable results owing to the high rate of culture failure (CF), reducing its reproducibility.[10,12] With advancements in genomic analysis techniques, next-generation sequencing (NGS) has emerged as an increasingly utilized method for POC chromosomal analysis.[13] Unlike G-banding, NGS requires only a small sample, does not need a sterile collection procedure, and can be performed on frozen or degraded tissues.[14] However, NGS also has limitations, such as its inability to detect polyploidy or balanced structural abnormalities.[15]

To date, only a few studies have directly compared the clinical utility of G-banding and NGS for POC chromosomal analysis, and statistical evidence remains limited.[13,16] In Japan, G-banding for POC chromosomal analysis is covered by health insurance, whereas NGS, despite its potential advantages, remains uninsured. To address this gap, we conducted a clinical study to compare G-banding and NGS for POC chromosomal analyses under Advanced Medical Care A. We aimed to evaluate the clinical utility of NGS in comparison with G-banding for presuming the causes of miscarriage and stillbirth.

## 2 | MATERIALS AND METHODS

### 2.1 | Study design and participants

This prospective cohort study was conducted in collaboration between two facilities. POC samples were collected between November 2022 and December 2023. In all cases, chromosomal analysis of the collected POC samples was performed using G-banding and NGS on the same POC specimens.

Inclusion criteria included: (1) Patients with a history of one or more spontaneous miscarriages diagnosed with clinical miscarriage in the current pregnancy before 12 weeks of gestation, and (2) patients diagnosed with clinical miscarriage (occurring at or after 12 weeks of gestation) or stillbirth (defined as pregnancy loss at 22 weeks of gestation or beyond) in the current pregnancy, regardless of a history of prior miscarriage. Cases in which the fetus or chorionic villi were naturally expelled or surgically collected at other facilities were subsequently brought to the participating study facilities. Until the transfer, these POC samples were stored under refrigeration (2–8°C) or at room temperature, depending on the handling conditions at the originating facility. POC samples collected under these criteria were analyzed using both G-banding and NGS.

### 2.2 | Sample collection and analysis methods

POC samples were rinsed with saline and processed under a stereomicroscope to minimize the risk of maternal cell contamination (MCC) by carefully removing maternal blood and decidual tissue. For G-banding analysis, ~100 mg of the sample was immediately immersed in the culture medium after collection. Concurrently, for NGS analysis, ~10 mg of the sample was either preserved in a Sample Protector for RNA/DNA (Takara Bio Inc., Shiga, Japan) or snap-frozen in liquid nitrogen and stored at −80°C.

The G-banding analysis was performed at a clinical laboratory facility registered with the Japan Association of Clinical Laboratory Systems. POC samples were incubated in the culture medium at 37°C for 72 h and subsequently treated with 20 mg/mL colcemid. Following sample preparation, the band patterns were analyzed in accordance with the guidelines of the International System for Human Cytogenomic Nomenclature.

For the NGS analysis, genomic DNA (gDNA) was extracted from POC samples using the NucleoSpin Tissue (Takara Bio, Shiga, Japan). Quant dsDNA BR Assay Kit (Invitrogen, Carlsbad, CA, USA) and agarose gel electrophoresis were used to evaluate the quality and integrity of the extracted gDNA. Whole-genome amplification (WGA) of gDNA was performed using the Embgenix PGT-A Kit (Takara Bio Inc., Shiga, Japan), followed by DNA library construction and low-coverage whole-genome sequencing (lcWGS) using the MiSeq System (Illumina Inc., San Diego, CA, USA). Sequencing data were analyzed using the Embgenix analysis software version 1.0.9j (Takara Bio Inc., Shiga, Japan) to detect copy number variations (CNVs). The

software identified CNVs of 8 Mb or larger and detected mosaicism at levels starting at 30%.

## 2.3 | Interpretation of results

Chromosomal abnormalities were defined differently for G-banding and NGS. In G-banding, they were identified when numerical or structural abnormalities in chromosomes were detected. However, in NGS, they included significant increases or decreases in the copy numbers of autosomes or sex chromosomes. Furthermore, changes in copy number below 30% were not considered significant, whereas changes of 80% or more were considered significant.

The cause of miscarriage or stillbirth was classified as "presumed" when chromosomal abnormalities with numerical aberrations were detected (fetal factors) or when either a normal karyotype other than 46,XX, or a balanced translocation was identified (maternal factors). Conversely, the cause was classified as "not presumed" if chromosomal analysis results were unavailable or if the result was a normal female karyotype (46,XX) due to the difficulty of excluding MCC.

## 2.4 | Statistics

The primary outcome was the rate of presumed causes of miscarriage or stillbirth determined using G-banding or NGS among all cases. Cases with failed chromosomal analysis were treated as those in which the cause could not be presumed. Furthermore, the secondary outcomes included (1) the rate of successful chromosomal analysis among all cases in which POC samples were submitted for analysis and (2) the rate of presumed causes of miscarriage or stillbirth for cases where chromosomal analysis was successfully performed using G-banding and NGS. These rates were compared between the two methods using McNemar's test with a one-sided significance level of 5%.

# 3 | RESULTS

We included 40 patients in this study, with an average maternal age of 36.3 ± 4.0 years (± standard deviation). Three patients (7.5%) had no prior history of miscarriage. These cases consisted of two clinical miscarriages (occurring after 12 weeks of gestation) and one stillbirth in their current pregnancy. During the study period, 34 patients (85.0%) experienced miscarriages before 12 weeks of gestation. POC samples were collected via D&E and spontaneous passage of tissues per vagina in 27 (67.5%) and 13 (32.5%) patients, respectively. The time (days) from POC sample retrieval (either through natural expulsion or surgical collection) to preservation was as follows: day 0, 15 cases (37.5%); day 1, eight cases (20.0%); day 2, two cases (5.0%); day 3, nine cases (22.5%); day 4, three cases (7.5%); and day 5 or later, three cases (7.5%). Fresh samples were used for 39 patients (97.5%), whereas frozen samples were used for one patient (2.5%) (Table 1). CF occurred in 13 patients with G-banding, and the rates of CF in relation to days from POC retrieval were as follows: day 0, three cases (20.0%); day 1, one case (12.5%); day 2, two cases (100%); day 3, three cases (33.3%); day 4, one case (33.3%); and day 5 or later, three cases (100%) (Table 2).

**TABLE 1** Demographic and clinical characteristics of the cohorts.

| | Patients with miscarriage and stillbirth ($n = 40$) |
|---|---|
| Age (years; mean ± SD) | 36.3 (±4.0) |
| Previous pregnancies (*n* (%)) | |
| 0 | 3 (7.5) |
| ≥1 | 37 (92.5) |
| Previous miscarriage and stillbirth (*n* (%)) | |
| 0 | 3 (7.5) |
| ≥1 | 37 (92.5) |
| Gestational age (weeks) (*n* (%)) | |
| < 12 | 34 (85.0) |
| ≥12 | 6 (15.0) |
| Method of POC collection (*n* (%)) | |
| D&E | 27 (67.5) |
| Natural expulsion | 13 (32.5) |
| The time (days) from POC retrieval to preservation in storage solution or freezing (*n* (%)) | |
| 0 | 15 (37.5) |
| 1 | 8 (20.0) |
| 2 | 2 (5.0) |
| 3 | 9 (22.5) |
| 4 | 3 (7.5) |
| ≥5 | 3 (7.5) |
| The preservation status of POC (*n* (%)) | |
| Fresh | 39 (97.5) |
| Frozen | 1 (2.5) |

Abbreviations: D&E, dilation and evacuation; POC, products of conception.

G-banding and NGS were used to perform chromosomal analysis in all cases, and the results were compared (Table 3). The distribution of chromosomal numerical abnormalities detected by NGS was as follows: four cases of trisomy 16 or mosaic trisomy 16; four cases of trisomy 22 or mosaic trisomy 22; four cases of sex chromosome aneuploidy (SCAs) comprising monosomy X (45,X) and a combination of partial trisomy and partial monosomy of chromosome X; three cases of trisomy 15; two cases of trisomy 13 or mosaic trisomy 13; two cases of trisomy 21; and one case each of trisomy of chromosome 7, 10, 18, and 20 (Table 3, Figure 1). In comparison, G-banding detected: three cases of trisomy 16, three cases of trisomy 22, two cases of monosomy X (45,X), trisomy 16, and one case each of trisomy 13, 14, 15, and 20. For balanced chromosomal translocations, G-banding identified one case of a Robertsonian translocation not detected by NGS. No cases of polyploidy were observed (Table 3).

**TABLE 2** The rate of culture failure in relation to days from POC retrieval.

| Days from POC retrieval to preservation in storage solution (*n*) | Patients with CF in G-banding<br>The rate of CF (*n* (%)) |
|---|---|
| 0 (*n* = 15) | 3 (20.0) |
| 1 (*n* = 8) | 1 (12.5) |
| 2 (*n* = 2) | 2 (100) |
| 3 (*n* = 9) | 3 (33.3) |
| 4 (*n* = 3) | 1 (33.3) |
| ≥5 (*n* = 3) | 3 (100) |

Abbreviations: CF, culture failure; POC, products of conception.

The primary outcome was the rate of presumed cause of miscarriage or stillbirth among all samples submitted using G-banding or NGS. NGS significantly outperformed G-banding in this regard (75.0% [30/40] vs. 42.5% [17/40], $p < 0.01$) (Table 4). Regarding the success rate of the chromosomal analysis, G-banding exhibited a CF rate of 32.5% (13/40), making analysis impossible in these cases. In contrast, NGS achieved a 100% (40/40) success rate ($p < 0.01$) (Table 4). Among the successfully analyzed cases, NGS presumed the cause in 70.3% (19/27) of the cases, compared with 62.9% (17/27) for G-banding ($p = 0.31$) (Table 5). In a subgroup analysis, NGS presumed the cause in 73.5% (25/34) of cases (miscarriages before 12 weeks of gestation), compared with 44.1% (15/34) for G-banding ($p < 0.01$). For miscarriages or stillbirths at 12 weeks or later, NGS presumed the cause in 83.3% (5/6) of cases, while G-banding presumed the cause in 33.3% (2/6) ($p = 0.13$) (Table 6). When the analysis was limited to fresh samples, NGS presumed the cause of miscarriage in 74.4% (29/39) of cases, which was significantly higher than the 43.6% (17/39) of G-banding ($p < 0.01$) (Table 7).

## 4 | DISCUSSION

In this study, we compared chromosomal analysis results for miscarriages or stillbirths using G-banding and NGS. NGS demonstrated superior efficacy compared with G-banding. G-banding was hindered by CF, limiting its ability to analyze all samples, whereas NGS successfully analyzed all submitted samples. This distinction highlights the robust adaptability of NGS, particularly for analyzing samples under varying conditions, such as prolonged storage periods. These findings reinforce the clinical significance of NGS in providing insights into the causes of miscarriages or stillbirths, ultimately offering a more reliable tool for patient management and care.

Miscarriage and stillbirth can result from various factors classified as either fetal or maternal. More than half of miscarriages are attributed to fetal chromosomal abnormalities.[6] Therefore, POC chromosomal analysis is a critical initial step in identifying the cause of miscarriage. Maternal factors include genetic abnormalities, uterine malformations, endocrine disorders, immune diseases, and thrombotic disorders.[7] However, conducting recurrent pregnancy loss (RPL)-related testing requires significant time and financial resources, owing to the wide range of diagnostic items involved. Popescu et al.[8] reported that the majority of women with a history of two pregnancy losses who underwent POC chromosomal microarray analysis that was abnormal had a normal result on RPL-related testing recommended by the American Society for Reproductive Medicine (74.6%). This finding highlights the cost-effectiveness and efficiency of prioritizing POC analysis from the second miscarriage onward, potentially reducing the need for comprehensive RPL-related testing and facilitating future pregnancy attempts. Conversely, when POC chromosomal analysis revealed normal fetal chromosomes, 85% of patients exhibited abnormalities in RPL-related testing, emphasizing the need for active investigation of maternal causes using RPL-related tests, particularly in cases when POC chromosomal analysis identifies a normal karyotype.[8] In this study, we compared the diagnostic efficacies of G-banding and NGS for presuming the causes of miscarriages or stillbirths. The overall rate of presumed causes using NGS was 75.0%, which was significantly higher than the 42.5% rate achieved using G-banding, demonstrating NGS's superior clinical utility across diverse POC sample conditions.

Furthermore, G-banding demonstrated a high rate of CF (32.5%). Previous reports indicate that 5%–38% of G-banding analyses fail because of unsuccessful cell culture, with success rates varying depending on the sample conditions, such as tissue degradation, improper sample collection, and delayed tissue retrieval[10,17–20] Additionally, the inherently non-sterile nature of vaginally collected POC samples increases the likelihood of CF[11] In this study, no restrictions were placed on the methods of POC sample collection, preservation, or the duration of storage. Consequently, a proportion of the included samples were likely to be in suboptimal conditions, which could explain the higher CF rates observed. In clinical practice, there are cases where immediate submission of POC samples for testing is not possible, such as when the decision to conduct testing is delayed or when a naturally expelled POC sample cannot be promptly transported to the institution. Furthermore, maintaining optimal cleanliness during sample collection may not always be feasible. Therefore, we incorporated these cases into our study. However, if samples were collected and preserved in a fresh state on the same day, such as immediately following D&E, the significant difference between NGS and G-banding might not have been observed. In contrast, NGS allows chromosomal analysis regardless of the condition of the POC samples. Unlike G-banding, NGS does not require cell culture and enables both qualitative and quantitative DNA analyses[13] For instance, in one case, chromosomal analysis was successfully performed on a frozen sample from a patient who could not immediately decide whether to undergo POC testing. Genomic diagnosis using NGS demonstrated its advantages by enabling the chromosomal analysis to be performed at a later time, providing flexibility for patients unable to make immediate decisions following miscarriage or stillbirth. Additionally, our previous study reported a successful chromosomal analysis of a vanishing twin syndrome case at 8 weeks of gestation, performed at the time of delivery at 40 weeks of gestation[21] This further highlights NGS's ability to analyze chromosomal abnormalities even in samples preserved for

**TABLE 3** Summary of chromosomal analysis using G-banding and NGS.

| No | Maternal age | Gestational weeks | G-banding | NGS | Method of POC collection | The time from POC retrieval to preservation in storage solution or freezing | The preservation status of POC |
|---|---|---|---|---|---|---|---|
| 1 | 32 | 8 | 45,X | 45,X | D&E | 0 | Fresh |
| 2 | 41 | 7 | CF | 47,XX,+15 | D&E | 0 | Fresh |
| 3 | 31 | 9 | 46,XY | 46,XY | D&E | 0 | Fresh |
| 4 | 32 | 14 | 46,XX | 46,XX | NE | 3 | Fresh |
| 5 | 31 | 7 | 46,XX | 46,XX | NE | 4 | Fresh |
| 6 | 35 | 8 | 47,XX,+14 | 46,XX | D&E | 0 | Fresh |
| 7 | 33 | 36 | 46,XY | 46,XY | NE | 4 | Fresh |
| 8 | 35 | 7 | 46,XX | 46,XX | D&E | 0 | Fresh |
| 9 | 41 | 8 | 47,XY,+16 | 47,XY,+16 | D&E | 0 | Fresh |
| 10 | 37 | 6 | 47,XY,+16 | 47,XY,+16 mosaic | D&E | 1 | Fresh |
| 11 | 40 | 10 | 47,XY,+22 | 47,XY,+22 | NE | 3 | Fresh |
| 12 | 39 | 7 | CF | 47,XY,+16 | NE | 0 | Fresh |
| 13 | 42 | 11 | CF | 46,XX | D&E | 6 | Fresh |
| 14 | 38 | 7 | 46,XY | 46,XY | D&E | 3 | Fresh |
| 15 | 30 | 15 | CF | 46,XY | NE | 0 | Frozen |
| 16 | 36 | 12 | 46,XX | 47,XX,+18 | NE | 0 | Fresh |
| 17 | 37 | 9 | 47,XX,+16 | 47,XX,+16 | D&E | 3 | Fresh |
| 18 | 41 | 8 | CF | 47,XY,+22 | D&E | 9 | Fresh |
| 19 | 42 | 8 | 47,XY,+13 | 47,XY,+13 | D&E | 3 | Fresh |
| 20 | 39 | 9 | CF | 47,XY,+21 | D&E | 3 | Fresh |
| 21 | 34 | 13 | 46,XY | 46,XY | NE | 1 | Fresh |
| 22 | 40 | 8 | 46,XX | 47,XY,+7 | D&E | 1 | Fresh |
| 23 | 34 | 8 | 46,XX,+13,der(13;14)(q10;q10) | 47,XX,+13 mosaic | NE | 0 | Fresh |
| 24 | 44 | 8 | 46,XX | 46,XX | D&E | 1 | Fresh |
| 25 | 31 | 8 | 46,XX | 46,XX | NE | 1 | Fresh |
| 26 | 40 | 8 | 47,XX,+22 | 47,XX,+22 mosaic | D&E | 0 | Fresh |
| 27 | 37 | 14 | CF | 46,XY | NE | 5 | Fresh |
| 28 | 31 | 8 | 47,XX,+22 | 47,XX,+22 | NE | 1 | Fresh |
| 29 | 39 | 8 | 46,XX | 46,XX | NE | 0 | Fresh |
| 30 | 35 | 8 | CF | 46,XX | D&E | 1 | Fresh |
| 31 | 28 | 8 | 46,XX | 46,XX | D&E | 1 | Fresh |
| 32 | 36 | 7 | CF | X segmental trisomy mosaic, X segmental monosomy | D&E | 3 | Fresh |
| 33 | 31 | 7 | 46,XX | 47,XX,+10 | D&E | 0 | Fresh |
| 34 | 35 | 6 | CF | 46,XY | D&E | 2 | Fresh |
| 35 | 35 | 6 | CF | 47,XX,+21 | D&E | 4 | Fresh |
| 36 | 37 | 8 | 45,X | 45,X | D&E | 3 | Fresh |
| 37 | 32 | 8 | CF | 45,X | D&E | 2 | Fresh |
| 38 | 38 | 8 | CF | 47,XY,+15 | D&E | 3 | Fresh |
| 39 | 39 | 9 | 47,XX,+20 | 47,XX,+20 | D&E | 0 | Fresh |
| 40 | 42 | 8 | 47,XY,+15 | 47,XY,+15 | D&E | 0 | Fresh |

Abbreviations: CF, culture failure; D&E, dilation and evacuation; NE, natural expulsion; NGS, next-generation sequencing; POC, products of conception.

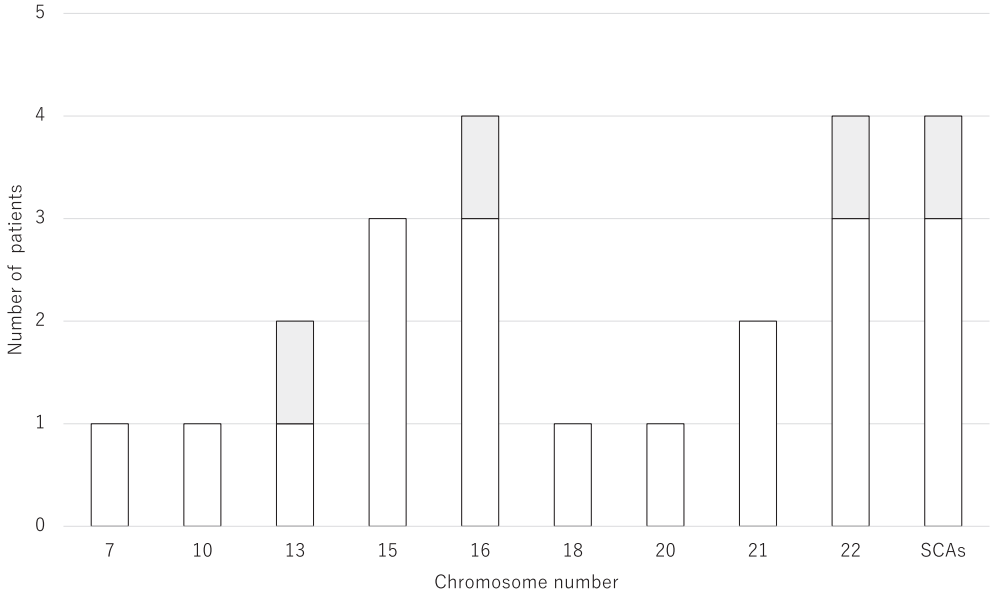


**FIGURE 1** Chromosomal abnormalities detected by NGS and the corresponding number of patients. The most frequently observed abnormalities were trisomy 16, trisomy 22 (including mosaic cases), and sex chromosome aneuploidies (SCAs), each identified in four cases. Among the SCAs, there were three cases of 45,X monosomy and one case of a combination of partial trisomy and partial monosomy of the X chromosome. White bars represent aneuploidy, and gray bars represent mosaicism.

**TABLE 4** Comparison of NGS and G-banding in presuming the cause of miscarriage among 40 cases.

| Patients with miscarriage and stillbirth ($n=40$) | G-banding | NGS | $p$-Value |
|---|---|---|---|
| Presumed cause of miscarriage | | | |
| Succeeded ($n$ (%)) | 17 (42.5) | 30 (75.0) | <0.01 |
| Chromosomal abnormalities ($n$ (%)) | 13 (32.5) | 23 (57.5) | <0.01 |
| 46,XY ($n$ (%)) | 4 (10.0) | 7 (17.5) | 0.375 |
| Failed ($n$ (%)) | 23 (57.5) | 10 (25.0) | <0.01 |
| 46,XX ($n$ (%)) | 10 (25.0) | 10 (25.0) | 1 |
| CF ($n$ (%)) | 13 (32.5) | 0 (0) | <0.01 |

Abbreviations: CF, culture failure; NGS, next-generation sequencing.

**TABLE 5** Comparison of NGS and G-banding in successfully analyzed cases.

| Successfully analyzed cases ($n=27$) | G-banding | NGS | $p$-Value |
|---|---|---|---|
| Presumed cause of miscarriage | | | |
| Succeeded ($n$ (%)) | 17 (62.9) | 19 (70.3) | 0.31 |
| Chromosomal abnormalities ($n$ (%)) | 13 (48.1) | 15 (55.5) | 0.31 |
| 46,XY ($n$ (%)) | 4 (14.8) | 4 (14.8) | 1 |
| Failed ($n$ (%)) | 10 (37.0) | 8 (29.6) | 0.31 |
| 46,XX ($n$ (%)) | 10 (37.0) | 8 (29.6) | 0.31 |

Abbreviation: NGS, next-generation sequencing.

extended periods. Our study inferred that CF likelihood in G-banding significantly increased with longer intervals between POC sample retrieval and processing, particularly beyond day 1. This underscores the importance of timely sample processing and preservation to minimize CF rates in G-banding analysis. In contrast, cases in which G-banding failed because of CF were successfully analyzed using NGS, highlighting the practical advantages of NGS over G-banding in clinical settings, especially in cases involving challenging sample conditions or delayed patient decision-making.

POC chromosomal analysis is significant in determining the causes of miscarriage and stillbirth. However, there is currently no definitive treatment for fetal chromosomal abnormalities in ongoing cases of pregnancy loss. When chromosomal abnormalities originating from the couple, such as balanced translocations, are detected, preimplantation genetic testing (PGT) is recommended for subsequent pregnancies. PGT for structural rearrangement (PGT-SR) does not increase the cumulative pregnancy rate; however, it can reduce the risk of recurrent miscarriages and provides valuable guidance for managing future pregnancies.[22] Additionally, patients who have experienced miscarriages often face significant anxiety about subsequent pregnancies. Determining the cause of miscarriage can

alleviate this anxiety and is considered effective for providing psychological support and managing subsequent pregnancies, helping patients approach subsequent pregnancies more positively.[8,23] In Japan, eligibility for PGT for aneuploidy and PGT-SR is determined by the Japan Society of Obstetrics and Gynecology (JSOG) and includes the following criteria: (1) a history of two or more implantation failures, (2) a history of two or more miscarriages or stillbirths, and (3) confirmed chromosomal structural abnormalities (such as balanced translocations) in the patient or their partner.[24,25] Therefore, not all patients who wish to undergo PGT based on their POC results are eligible for their next pregnancy. Moreover, even if patients meet the JSOG criteria, PGT requires assisted reproductive technologies (ART). This means that patients who could otherwise conceive naturally may need to undergo ART solely for PGT, which may not always be in their best interest or provide significant clinical benefits.

Our study has some limitations. First, a normal female karyotype (46,XX) was classified as "not presumed" due to the difficulty in excluding MCC. If MCC could be ruled out in 46,XX cases, further classification into "presumed" and "not presumed," would be possible. In this study, both G-banding and NGS classified cases with a 46,XX result as "not-presumed," and statistical analyses were conducted with this classification applied to both groups, ensuring the validity of our methodology. Our NGS approach focused on detecting CNVs; however, incorporating additional genetic testing methods, such as short tandem repeat analysis, could enable MCC detection, thereby enhancing diagnostic accuracy.[26] Furthermore, the inclusion of quantitative fluorescence PCR or single-nucleotide polymorphism analysis could facilitate the identification of balanced translocations and polyploidy for specific types of chromosomal abnormalities, which are typically difficult to differentiate using standard NGS.[15,27] These additions may help overcome the inherent limitation of relying solely on CNV analysis. In contrast, G-banding can detect polyploidy and balanced translocations and is also less costly compared with NGS. Therefore, from a health care economic perspective, G-banding may be a preferable choice, depending on sample conditions and institutional resources.

Among the cases in which NGS detected CNVs, trisomy 16 and 22 were the most frequently observed abnormalities, which is consistent with previous reports.[13,15] These were followed by SCAs, including a complex mosaic involving partial trisomy of the long arm and partial monosomy of the short arm of chromosome X. G-banding identified one case of trisomy 13 with a Robertsonian translocation (case 23), suggesting the possibility of a Robertson translocation carrier in one of the partners, a feature undetectable by NGS. Subsequent karyotype analyses of both partners revealed normal chromosomal structures with no balanced translocations. This result highlights one of the limitations of NGS, which is its reduced effectiveness in detecting structural chromosomal abnormalities, such as balanced translocations. Additionally, tissues were collected from the same POC sample for both G-banding and NGS; however, the specific regions of the samples used were not completely identical. A previous study reported that chromosomal analysis results can vary depending on the sampling location within the POC tissue, with mosaics observed in the chorionic villi in 17% of cases, introducing potential sampling bias during specimen collection.[28] This limitation may explain the discrepancies observed in certain cases (such as Case 10 and Case 26) where G-banding and NGS produced differing results despite both being classified as "presumed." Conversely, in Cases 6, 16, 22, and 33, in which one method identified 46,XX, it remains uncertain whether MCC contributed to these discrepancies. The current study design does not allow for definitive conclusions regarding these cases.

Finally, only 15 cases had a sample retrieval-to-preservation time of Day 0. This is due to the infrastructure of the Japanese medical system, where not all facilities are equipped to conduct G-banding or POC-NGS. Consequently, our study design permitted sample submissions from external facilities, leading to variability in sample handling and preservation times, particularly affecting the evaluation of the usefulness of G-banding.

**TABLE 6** Comparison of miscarriages and stillbirths occurring before 12 weeks and at or after 12 weeks for which the cause could be presumed using G-banding and NGS.

| | G-banding | NGS | *p*-Value |
|---|---|---|---|
| Patients with miscarriage occurring before 12 weeks ($n=34$) | | | |
| Succeeded (*n* (%)) | 15 (44.1) | 25 (73.5) | <0.01 |
| Patients with miscarriage or stillbirth occurring at or after 12 weeks ($n=6$) | | | |
| Succeeded (*n* (%)) | 2 (33.3) | 5 (83.3) | 0.13 |

Abbreviation: NGS, next-generation sequencing.

**TABLE 7** Comparison of NGS and G-banding in analysis limited to fresh samples.

| Analysis limited to fresh samples ($n=39$) | G-banding | NGS | *p*-Value |
|---|---|---|---|
| Presumed cause of miscarriage | | | |
| Succeeded (*n* (%)) | 17 (43.6) | 29 (74.4) | <0.01 |
| Chromosomal abnormalities (*n* (%)) | 13 (33.3) | 23 (59.0) | <0.01 |
| 46,XY (*n* (%)) | 4 (10.2) | 6 (15.4) | 0.25 |
| Failed (*n* (%)) | 22 (56.4) | 10 (25.6) | <0.01 |
| 46,XX (*n* (%)) | 10 (25.6) | 10 (25.6) | 1 |
| CF (*n* (%)) | 12 (30.8) | 0 (0) | <0.01 |

Abbreviations: CF, culture failure; NGS, next-generation sequencing.

## 5 | CONCLUSION

NGS demonstrated superior efficacy over G-banding in presuming the cause of miscarriage or stillbirth in patients who experience pregnancy loss by accommodating diverse POC sample conditions and preservation states. These findings highlight its clinical value, particularly in challenging scenarios involving degraded tissues, improper sample collection, delays in tissue retrieval, and situations where immediate patient decision-making is difficult. As a reliable tool, it enhances diagnostic accuracy and contributes to improved management of miscarriages and stillbirths.

### ACKNOWLEDGMENTS

The authors thank Takara Bio Inc. for invaluable technical support and expertise throughout this research. Their dedication to excellence and collaborative spirit have been instrumental in overcoming challenges and achieving meaningful results. We would like to thank Editage (www.editage.jp) for English language editing.

### CONFLICT OF INTEREST STATEMENT

The corresponding author (T.T.) is employed by a department funded by Takara Bio, which also provided financial support for this research. This affiliation may be perceived as a potential conflict of interest. All other authors declare no conflicts of interest.

### DATA AVAILABILITY STATEMENT

All data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding authors.

### ETHICS STATEMENT

The study was approved by the Osaka University Medical School Hospital Observation Research Ethics Review Committee (Approval No.: 21434).

### INFORMED CONSENT

Written informed consent was obtained from all the patients to participate in this study, and for their anonymized information to be published in this article.

### ANIMAL STUDIES

This article does not contain any studies with animal subjects performed by any of the authors.

### CLINICAL TRIAL REGISTRY SUBSECTIONS

This study does not contain data from the clinical trial registry.

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**How to cite this article:** Honda H, Takiuchi T, Handa M, Wakui N, Tsuji S, Goto T, et al. Comparative prospective study on the clinical utility of G-banding and next-generation sequencing for chromosomal analysis of products of conception under Advanced Medical Care A in Japan. Reprod Med Biol. 2025;24:e12655. https://doi.org/10.1002/rmb2.12655