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TECHNOLOGICAL INNOVATIONS

Molecular analysis of products of conception obtained by hysteroembryoscopy from infertile couples

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Abstract
Purpose To analyze the molecular cytogenetic data obtained from products of conception (POC) obtained by selective biopsy of first trimester miscarriages and to estimate the rate of chromosomal anomalies in miscarriages from pregnancies achieved by natural conception (NC) or by assisted reproductive technology (ART) interventions.
Methods We used KaryoLite™ BoBs™ (PerkinElmer LAS, Wallac, Turku, Finland) technology to analyze 189 samples from ART or NC pregnancies.
Results All POC were successfully evaluated. A higher incidence of chromosomal abnormalities was observed in POC after ART using the patient's own oocytes than from NC pregnancies (62.7 % vs. 40.6 %; $p<0.05$). The lowest incidence of chromosomal abnormalities was observed in POCs ART using donor eggs from women younger than 35 years (12.8 %). No statistical differences in the percentage of abnormal miscarriages were observed in correlation with sperm concentration: a sperm concentration less than 5 million/mL produced 75 % abnormal results and a concentration higher than 5 million/mL produced 51 %.
Conclusions POC analysis is essential to determine the cause of pregnancy loss. Using culture-independent molecular biology techniques to analyze POCs avoids limitations such as growth failure and reduces the time required for analysis. Selective biopsy of fetal tissue by hysteroembryoscopy avoids the risk of misdiagnosis due to maternal cell contamination. Our results show that maternal age, sperm quality, and ART-assisted pregnancies are risk factors for abnormal gestations.

Keywords Products of conception · Hysteroembryoscopy · Assisted reproductive technology · Karyolite BoBs · Aneuploidy

Capsule POC analysis is essential to determine the cause of pregnancy loss. Using molecular tecnique, Karyolite BoBs, avoid growth failure and reduce time requires for analysis. Selective biopsy of fetal tissue by hysteroembryoscopy avoids the risk of misdiagnosis due to maternal cell contamination.

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Introduction

Miscarriages are the most common complication during early pregnancy. Clinically recognized pregnancy loss occurs in approximately 15–25 % of pregnancies, with most occurring in the first trimester [1]. Although there are many known causes and risk factors for early pregnancy loss, about 60 % of cases [2] are caused by sporadic chromosomal abnormalities [2–6] which are usually numerical (86 %) [7]. These cytogenetic anomalies include autosomal trisomies (27 %), polyploidies (10 %), chromosome X monosomy (9 %), and structural rearrangements (2 %) [8]; double trisomies, as well as multiple trisomies, are infrequent, with an incidence of about 0.7 % [9]. Recurrent pregnancy loss (RPL) is a clinical entity which is distinct from sporadic miscarriage [10] and is defined by two or more failed clinical pregnancies [11]. It is estimated that fewer than 5 % of women will experience two consecutive miscarriages, and only 1 % experience three or more [12]. RPL can be explained by many factors, such as genetic,

anatomic, endocrinological, and immunological abnormalities [13–15], however, the disease has an unexplained or idiopathic origin in more than 50 % of couples with RPL [16]. One possible cause is that these couples have an increased tendency to produce aneuploid embryos which then provoke more miscarriages. Marquard et al. (2010) found that 80 % of miscarriages occur in women older than 35 years and that many of these cases of RPL would have been unexplained if chromosome testing had not been carried out on the products of conception (POC) [17].

For these reasons cytogenetic evaluation of POC is essential to determine the cause of sporadic and recurrent pregnancy loss; it also allows the risk of recurrence to be estimated for future pregnancies, thus improving the chances of subsequently producing a healthy full-term pregnancy. If an anomaly is identified as the cause of the miscarriage this information can comfort the couple and alleviate their feelings of culpability [18]. Indeed, current American Society for Reproductive Medicine (ASRM) recommendations state that POC chromosome testing may be psychologically beneficial to patients and can aid treatment decisions in the setting of RPL [19]. Cytogenetic analysis by karyotyping tissue-cultured POC metaphase spreads has traditionally allowed the identification of chromosomal abnormalities larger than 5–10 Mb (including the number and arrangement) in spontaneous pregnancy losses. Sometimes no conclusive results can be obtained after traditional cytogenetic analysis; there are several reasons for this, including failure of POC culture growth, microbial infection, specimen maceration, suboptimal chromosome preparations, or poor chromosome morphology. Additionally, occasionally karyotypes other than 46,XX can incorrectly be called as 46,XX which may therefore underestimate any underlying fetal chromosome abnormalities if there is any maternal cell contamination (MCC) or if there is selection against chromosomally abnormal cells over longer-duration culture periods (around 15 days) [20]. Cytogenetic analysis of cells from curettages is vulnerable to selective overgrowth of maternally derived cells, and can falsely produce a normal female karyotype at rates of 29.0 to 89.7 % [21, 22] because of the analysis of predominant maternal cells instead of POC derived cells [21–23]. Therefore specimen selection is crucial and affects the MCC rate [24]. Another limitation of karyotyping POC is the resolution limit, which sometimes does not allow detection of submicroscopic deletions and/or duplications, although new molecular cytogenetic methods avoid some of these pitfalls and reduces the time required for analysis. For these reasons, different molecular approaches that are not culture dependent, such as microarray-based comparative genomic hybridization, fluorescence in situ hybridization (FISH), multiplex ligation-dependent probe amplification (MLPA), and quantitative fluorescent polymerase chain reaction (QF-PCR) have been proposed for genetic POC analysis [7, 25–29]. We used the KaryoLite™ BoBs™ assay to genetically analyze POC samples in this study. KaryoLite™ BoBs™ uses a total of two beads per arm to provide dosage information about terminal and pericentromeric chromosomal regions, and a total of three beads to provide dosage information on the pericentromeric, interstitial, and terminal regions of q-arms of chromosomes [18].

In this study we report the molecular cytogenetic data obtained from KaryoLite™ BoBs™ analysis of POC samples from first trimester miscarriages, comparing the rate of chromosomal anomalies in miscarriages from natural conception (NC) pregnancies to those achieved via assisted reproductive technologies (ART). To minimize MCC, our specimens were collected by hysteroembryoscopy, a technique which enables examination of the embryo in utero before it undergoes mechanical destruction during the evacuation procedure [10]. This technique allows selective samples to be obtained from the embryo or trophectoderm whilst avoiding MCC.

Materials and methods

Design

In this retrospective review, POC samples were received between March 2011 and December 2013 and analyzed using KaryoLite™ BoBs™ (PerkinElmer LAS, Wallac, Turku, Finland) assays. The laboratory received POC samples from a total of 189 pregnancies that arrested during the first trimester and which were obtained by selective biopsy after hysteroembryoscopy, following the protocol established by Ferro et al. [20] In most cases mainly embryo and trophectoderm tissues were obtained.

Results were analyzed depending on whether the pregnancy was achieved by NC or after ART. There were 157 (83.1 %) POC samples from patients that conceived using ART and 32 (16.9 %) from NC. In the ART group, 118 (75.2 %) of the miscarriages had occurred after ART treatment using the patient's own oocytes, and 39 (24.8 %) occurred after ovum donation cycles. POC samples derived from NC pregnancies were used as the control group. The effect of seminal quality on the incidence of chromosomal abnormalities was also evaluated.

Hysteroembryoscopy

Hysteroembryoscopy was performed following a routine protocol from Ferro et al. [20]. All hysteroembryoscopies and subsequent curettage procedures were performed under general anesthesia on an outpatient basis. A Hamou examination and contact hysteroscope III with a Hopkins 30° telescope (with magnifications 1x and 60x, diameter 2.9 mm, length 30 cm) and a 4.3 mm Bettocchi single-flow operating sheath, which allows the use of semi-rigid 5-Fr. operating instruments (Karl Storz GmbH & Co. KG, Tuttlingen, Germany), were used.

Normal saline was used as the distending medium. The hysteroscope was gently introduced into the uterine cavity without cervical dilatation and the gestational sac prominence was located. A small hole was made in the gestational sac wall using 5-Fr. biopsy spoon forceps (Karl Storz GmbH & Co. KG) and the scope was gradually introduced into the extracoelomic and amniotic cavities. Direct chorion and embryo biopsies were taken and placed in normal saline, and suction curettage was performed immediately afterward. A course of oral azithromycin was given postoperatively. POC samples were collected and transported in normal saline at room temperature.

KaryoLite™ BoBs™ technology

KaryoLite™ BoBs™ is a multiplex bead-based suspension array using microspheres that are internally dyed with a combination of two spectrally distinct infrared and red fluorochromes which can produce more than 100 specific spectrums. Each bead is coupled to DNA amplified from bacterial artificial chromosomes (BACs) and analyzed using a Luminex® cytometric acquisition system with two separate lasers (Luminex Corp., Austin, Texas) equipped with xPonent® 3.1 software (Perkin Elmer, Turku, Finland). Experiments with acceptable quality control parameters had more than 50 analyzed bead/BAC combinations alongside both male and female reference DNA samples [30].

Briefly, genomic DNA was extracted (QIAamp DNA Mini Kit, Qiagen, Inc., Chatsworth, CA, USA), labeled, purified, hybridized to BACs-on-Beads™ probes, bound to the reporter molecules (streptavidin-phycoerythrin), and washed. Thereafter the fluorescence signals were measured and the results were analyzed. Once the DNA was extracted, it was amplified with a primer solution, labeled by enzymatic incorporation of biotinylated nucleotides, and purified using a PCR purification kit. It was then incubated overnight with BAC clones attached to dyed beads, after which the hybridized beads were transferred onto a filter plate and washed again. Next the beads were incubated with a reporter that binds biotinylated DNA and were then washed and resuspended for measurement according to the manufacturer's protocol.

Figure 1 summarizes the main steps of the assay: 1) Obtaining genomic DNA; 2) Sample, and reference male and female, DNA labeling with biotinylated nucleotides; 3) Purification of labeled DNA; 4) Hybridization of the labeled DNA and purification with the BoBs mixture; 5) After serial washings, microsphere incubation with streptavidin-phycoerythrin which binds biotinylated DNA; 6) Bead washing and re-suspension and signal measurement.

The relative amount of DNA bound to the beads was determined using a Luminex® 100™/200™ instrument system with xPONENT® 3.1 and BoBsoft™ v2 analysis software that produces graphical ratio line-plots and a bar graph for each sample. A sample was defined as "duplicated/deleted" in a chromosome when the fluorescence in the test was higher/lower than that of the reference sample. Single copy gains and losses generate ratios ranging from 1.3 to 1.4 and from 0.6 to 0.8 respectively [31]. This protocol requires 24–48 h in the laboratory.

Statistics

The χ-square test and Fisher exact test ($p<0.05$) were used to compare study groups with respect to percentages. The Welch-test was used to compare non-categorical variables. Student's *t* test was used to compare categorical variables. A confidence interval of 95 % ($p<0.05$) was considered to be statistically significant.

Results

The description of the study population is shown in Table 1. The mean female age was 35.5±4.0 years in the case of NC, 37.0±3.3 years for ART-assisted pregnancies with the patient's own oocytes, and 39.7±3.2 years for oocyte recipients (the donor age was less than 35 years). We found statistically significant differences in age among patients with NC and women who underwent ART with their own oocytes ($p=0.031$). The mean male age was 37.2±4.1 years in the case of NC, 38.5±4.9 years in ART-assisted conception using the patient's own oocytes, and 41.8±6.4 years in ART with an oocyte donor. We found a statistically significant difference in age among patients that achieved a NC and women who underwent ART with donated oocytes ($p=0.0005$). The mean sperm concentration was 38.2±30.4 million/mL for ART using the patient's own oocytes and 33.8±25.7 million/mL

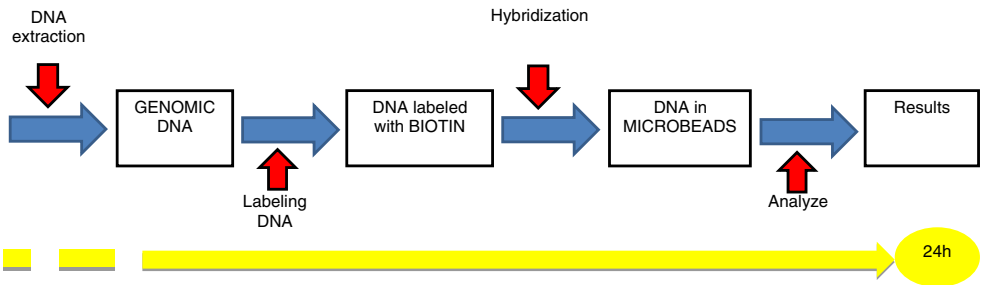


Fig. 1 KaryoLite™ BoBs™ assay flowchart

Table 1 Description of the sample population

	NC	ART: Own oocytes	ART: Donated oocytes	Total
No. of cases	32	118	39	189
Mean female age (± *SD*)	35.5±4.0*	37.0±3.3*	<35	37.3±3.7
Mean male age (± *SD*)	37.2±4.1**	38.5±4.9	41.8±6.4**	39.0±5.3
Mean no. previous miscarriages (± *SD*)[a]	–	0.3±0.7***	0.8±1.2***	0.6±1.0
Mean no. previous implantation failures (± *SD*)[a]	–	0.7±1.2****	1.2±1.4****	0.8±1.2
Mean semen concentration (± *SD*)	–	38.2±30.4	33.8±25.7	37.7±29.6
Mean gestational age (± *SD*)	8.2±1.4	8.3±1.0	8.2±0.9	8.3±1.0

NC natural conception, *ART* assisted reproduction technology

$P^{*,**,***,****} < 0.05$

[a] In cycles with donated oocytes, mean previous miscarriages and implantation failures corresponded to previous ART cycles using the patient's own oocytes

in ART with an oocyte donor. No statistically significant differences were observed between the mean gestational ages at the time of pregnancy loss for each group. In all the cases the pregnancy stopped before week 12.

Results according to the origin of gestation

As shown in Table 2, all POC samples were analyzed successfully. Of the 189 cases, 92 produced an abnormal result (48.7 %). No significant differences in the male/female ratio were observed in any of the groups studied: among the normal results, 50.5 % were 46,XX and 49.5 % were 46,XY. However, when we compared the percentage of abnormalities between NC and ART pregnancies using the patient's own oocytes there was a statistically significant difference (40.6 vs. 62.7 %; $p=0.0410$). Similarly, the percentage of abnormality between NC and pregnancies using ART with oocyte donation was also statistically significant (40.6 vs. 12.8 %; $p=0.0162$) as was the percentage of abnormal POC samples resulting from ART pregnancies using the patient's own oocytes versus donated oocytes (62.7 vs 12.8 %, $p=0.0078$).

Of the cases with abnormal results 78 (84.8 %) were trisomic and 14 (15.2 %) were monosomic, and of the former, 10 were from NC, 63 from pregnancies using the patient's own oocytes, and 5 with donated oocytes. No statistically significant differences in the incidence of trisomies were observed when comparing the three groups; however, 49.3 % of the trisomies involved chromosomes 22 and 16. Regarding the 14 monosomic POC samples, 3 were from NC and the rest were from ART-assisted gestations with the patient's own oocytes, although the difference was not statistically significant; no samples from ART with oocyte donation were monosomic. Eight of the monosomies were 45,X, two were 45,Y, two were monosomy 21, and two were monosomy 22. Unfortunately, this technique cannot discriminate between sex chromosome monosomies or haploid fetuses with only a maternal or paternal contribution.

Figure 2 shows aneuploidy analysis for each chromosome; notably, aneuploidy for chromosomes 1, 12 and 19 were not

Table 2 Results according to the origin of gestation

	NC	ART: OWN OOCYTE	ART: DONATED OOCYTE	TOTAL
No. of cases	32	118	39	189
Normal (%)	19 (59.4)	44 (37.3)	34 (87.2)	97 (51.3)
Normal XX (%)	8 (42.1)	22 (50.0)	19 (55.9)	49 (50.5)
Normal XY (%)	11 (57.9)	22 (50.0)	15 (44.1)	48 (49.5)
Abnormal (%)	13 (40.6)*,**	74 (62.7)*,***	5 (12.8)**,***	92 (48.7)
Trisomy (%)[a]	9 (69.2)	61 (82.4)	5 (100.0)	75 (81.5)
Double trisomy (%)[a]	1 (7.7)	2 (2.7)	0	3 (3.3)
Autosome monosomy (%)[a]	1 (7.7)	5 (6.8)	0	6 (6.5)
Sex chromosome monosomy (%)[a]	2 (15.4)	6 (8.1)	0	8 (8.7)

NC natural conception, *ART* assisted reproductive technology

$P^{*,**,***} < 0.05$

[a] Percentage of specific abnormalities related to the total number of abnormal POC

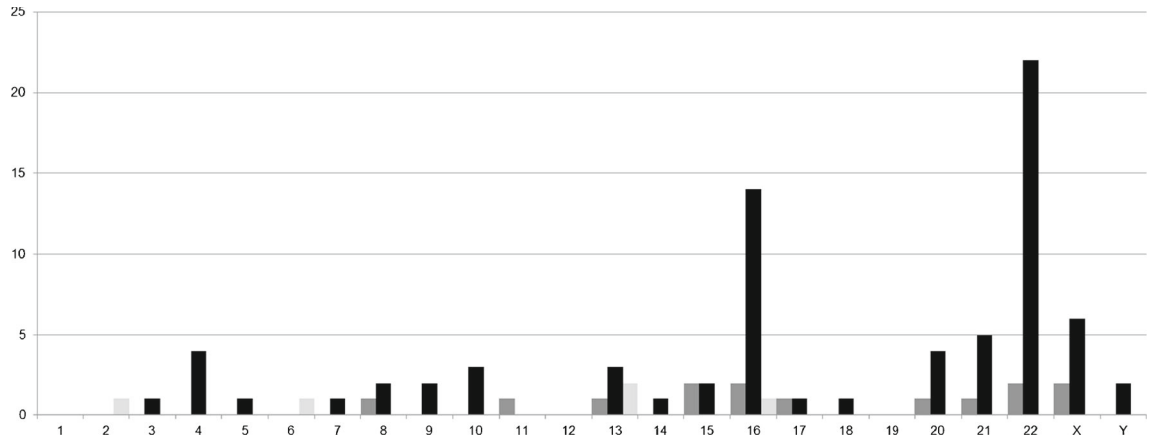


Fig. 2 Aneuploidy analysis by chromosome. *Dark grey*: NC. *Black*: ART with the patient's own oocyte. *Light grey*: ART with a donated oocyte

observed. In the NC group monosomy X, trisomy 15, trisomy 16, and trisomy 22 were the most frequent aneuploidies detected. In the ART group using their own oocytes trisomy 22 was the most common, followed by trisomy 16 and monosomy X. In the ART group with donated oocytes the most frequently detected aneuploidy was trisomy 13. In general, as shown in Table 3, the most frequent aneuploidies detected were trisomy 22 (22 cases), trisomy 16 (17 cases), and monosomy X (8 cases). The mean female age was significantly higher for trisomy 16 ($p=0.0177$) and trisomy 22 ($p=0.0110$) compared to those with monosomy X miscarriages. No statistically significant differences in the mean gestational age or sperm concentrations were observed between groups.

Results according to female age (Fig. 3)

Most (65.6 %) NC pregnancy miscarriages occurred between 35 and 41 years (Fig. 3a) with most trisomies occurring from 36 years, although monosomies appeared to be independent of female age. Miscarriages onset earlier in ART-assisted pregnancies using the patient's own oocytes (76.3 % occurred between 35 and 41 years), whereas trisomies and monosomies started to appear at 32 years (Fig. 3b). However, there was no relationship between female recipient age and aneuploidy in ART-assisted pregnancies with donated oocytes (Fig. 3c).

Table 3 The most frequent aneuploidies detected and sample population description

	45,X0	Trisomy 16	Trisomy 22
No. of cases	8	17	22
Mean gestational age (± *SD*)	8.9±1.7	8.1±0.7	8.5±1.1
Mean female age (± *SD*)	31.4±4.7*,**	37.6±2.8**	37.1±2.3*
Mean semen concentration (± *SD*)	39.0±30.2	48.0±34.7	33.8±21.8
≥2 Previous implantation failures	2	3	3
1 Previous miscarriage	1	0	0
≥2 Previous miscarriages	0	1	1

$P^{*,**}<0.05$

Results according to gestational age and sperm concentration

In the NC group, genetically normal pregnancies stopped from six to twelve weeks of pregnancy, most arresting at week seven, while monosomic and trisomic pregnancies progressed to week nine (Fig. 4a). In the ART group using the patient's own oocytes, genetically normal pregnancies stopped between week six to week nine but monosomic and trisomic pregnancies progressed up to the eleventh week (Fig. 4b). In the ART group using donated oocytes, genetically normal pregnancies stopped from week six to week eight but most of these pregnancies, as well as trisomic pregnancies with donated oocytes, arrested from week seven to eight (Fig. 4c).

No statistical differences in the percentage of genetically abnormal miscarriages were observed in correlation with sperm concentration: a sperm concentration of less than 5 million/mL produced 75 % abnormal results and a concentration higher than 5 million/mL resulted in 51 % abnormal results. Finally in a subset of 30 cases, material from the embryo or trophoblast was analyzed and results were similar in both tissues.

Discussion and conclusions

Analysis of early spontaneous miscarriages is essential to determine the cause of pregnancy loss and to counsel couples

appropriately about their reproductive risks and the possible outcomes, especially in an IVF setting [32]. Moreover, the most common cause of early pregnancy loss during the first trimester is chromosomal abnormality and so chromosomal analysis of the POC is important to classify chromosomally normal and abnormal miscarriages and to understand their causes. Until now, POC studies have been carried out using cell culture followed by conventional karyotyping. However when using these techniques the incidence of chromosomal abnormalities in miscarriages in the general population ranges between 40 and 80 %, depending on the culture methods adopted [33–36]. Proper chromosomal analysis of POC samples is not always feasible for several reasons which can include: cell culture growth failure (the failure rate in POC samples cultured after curettage ranges between 5 and 42 % [10]), suboptimal chromosome preparations, MCC, and a low resolution limit that does not allow the detection of submicroscopic deletions and duplications. Molecular biology techniques that are culture independent can avoid such limitations. In this study we selected the KaryoLite™ BoBs™ molecular technique which allowed us to detect aneuploidy in all the POC samples analyzed and to eliminate the need to tissue culture POC samples before studying them, thus reducing the time required to analyze them.

Conventional POC tissue culturing can produce a bias in the rate of abnormal karyotypes detected because decidua and maternal cell overgrowth can produce a false negative normal female karyotype [21]. Additionally, MCC after conventional curettage can also result in over-diagnosis of a normal 46,XX karyotype. For example, Lathi et al. combined 1222 first-trimester miscarriage specimens from a multi-center study, and using single-nucleotide polymorphism (SNP) microarray technology, estimated that the overall rate of MCC was 22 % [24]. Thus it was crucial for us to obtain non-MCC POC tissue samples for our KaryoLite™ BoBs™ genetic analyses. Therefore we obtained POC samples by hysteroembryoscopy which allowed us to perform a selective fetal tissue biopsy, thereby increasing the accuracy of our 46,XX results, and avoiding false negative misdiagnoses due to MCC.

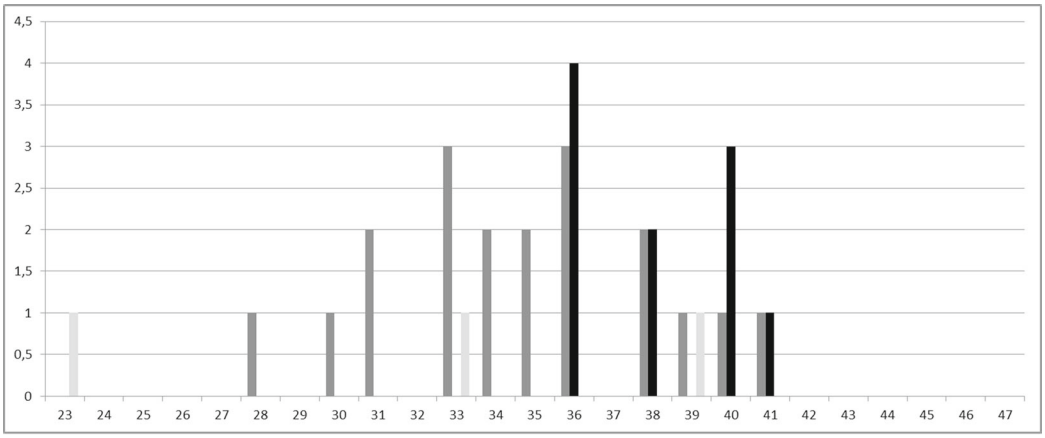
Hysteroembryoscopy also allows feto-placental mosaicisms (which sometimes cause miscarriages) to be detected, and allows different karyotypes to be discriminated in bichorionic–biamniotic twin miscarriages. Ferro et al. assessed the reliability of karyotypes obtained from spontaneous abortion curettage samples by comparing them with karyotypes obtained from selective embryonic and chorionic biopsies obtained by hysteroembryoscopy immediately before curettage. At least one of the specimens was successfully processed and karyotyped in 79.7 % (55/69) of the cases however, the samples failed to grow in 21 % of instances [20]. Moreover, using this molecular technique produced results after hysteroembryoscopy in 100 % of cases without obtaining any differences in male/female ratios. In our study, we analyzed two different tissue types (embryo and trophoblast) with different germ layer origins in the same 30 POC samples and found no differences between them.

The KaryoLite™ BoBs™ technique, which is based on DNA quantification, overcomes the growth failure limitation of traditional cytogenetics. However it cannot identify low grade mosaicisms, polyploidies, balanced arrangements, and small supernumerary marker chromosomes (sSMCs). Nevertheless, mosaic aneuploidies can be identified if the abnormal cell type is present in 50 % or more of the total number of cells. Similarly, MCC of up to 50 % cell cultures did not mask the detection of aneuploidies using traditional karyotype techniques [18]. Additionally, the incidence of tetraploidies and inherited chromosomal rearrangements is only 2 to 3 % in most studies [37]. Therefore we consider that the benefit of avoiding growth failure when using this molecular technique to the study POC samples outweighs its drawbacks.

In this study we successfully analyzed all the POC samples according to their gestational origin: NC, ART using the patient's own oocytes, and ART using donated oocytes. We observed that there was a considerably higher rate of aneuploidy in the ART group using the patient's own oocytes: which showed an abnormal karyotype in 62.7 % of the POC compared to 40.6 % in the NC cases. These results could be explained by differences in female age because the mean maternal age in the ART group using their own oocytes was significantly higher than that of the NC group. In the ART group using donated oocytes all donors were young women (younger than 35 years) and for these reasons the origin of the miscarriages was not usually chromosomal and the rate of abnormalities was the lowest. No relationship between aneuploidy and the donor recipient's age was observed. Regarding the effect of female age and the type of chromosomal abnormalities, autosomal trisomy was the most frequent finding in all three groups (average 84.8 %); chromosomes 22 and 16 were involved in 49.3 % of the trisomies, and 57.1 % were 45, X. Our data showed that mean female age in monosomy X miscarriages was significantly lower than for miscarriages with trisomy 16 and trisomy 22. Trisomies appeared from 36 years in NC pregnancies while in ART pregnancies using the patient's own oocytes they appeared from 32 years; interestingly, monosomies seemed to be independent of female

Fig. 3 Results according female age and gestation origin. **a**: Natural conception. *Dark grey*: Normal; *Black*: Trisomy; *Light grey*: Monosomy. **b**: ART using the patient's own oocyte. *Dark grey*: Normal; *Black*: Trisomy; *Light grey*: Monosomy. **c**: ART using a donated oocyte. *Dark grey*: Normal; *Black*: Trisomy; *Light grey*: Monosomy. The distribution of monosomies is independent of age in all three pregnancy type groups. Trisomies onset at an earlier age in the group of ART-assisted pregnancies using the patient's own oocyte compared to the NC group (32 years and 36 years respectively). In ovum donation there is no relationship between recipient age and the occurrence of trisomies

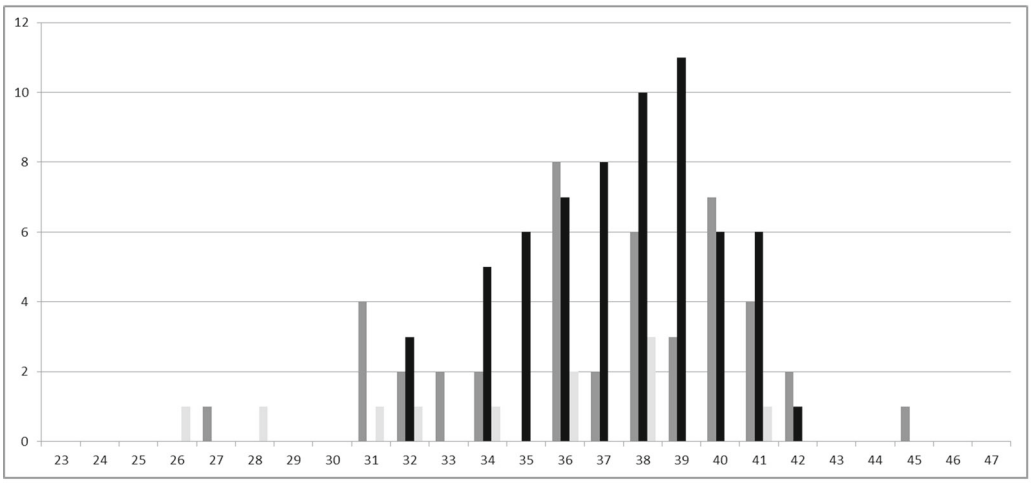
a



Natural conception

Dark grey: Normal; Black: Trisomy; Lightgrey: Monosomy.

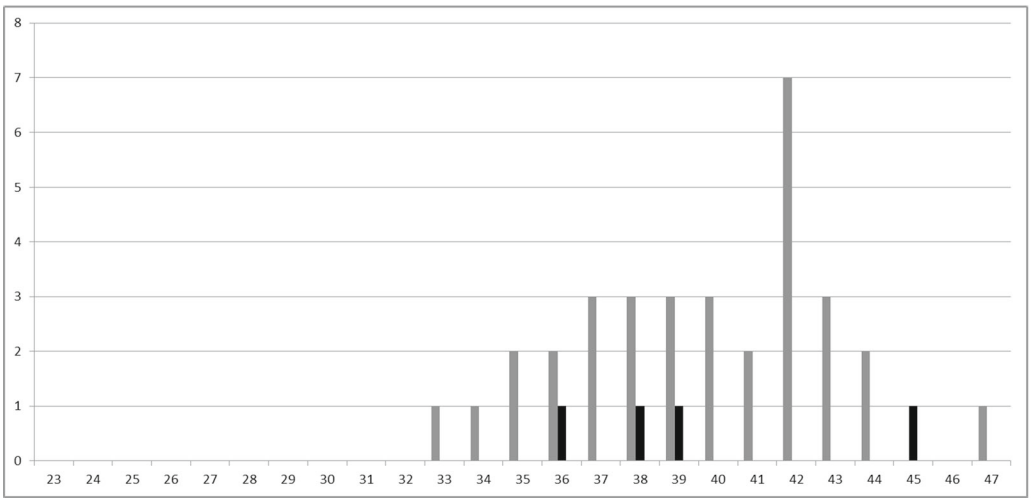
b



ART using the patient's own oocyte.

Dark grey: Normal; Black: Trisomy; Lightgrey: Monosomy.

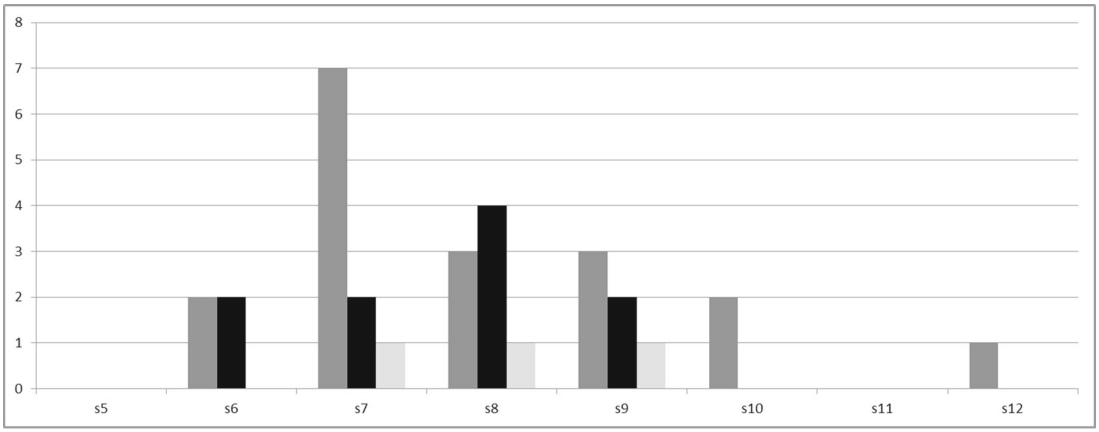
c



ART using a donated oocyte.

Dark grey: Normal; Black: Trisomy; Lightgrey: Monosomy.

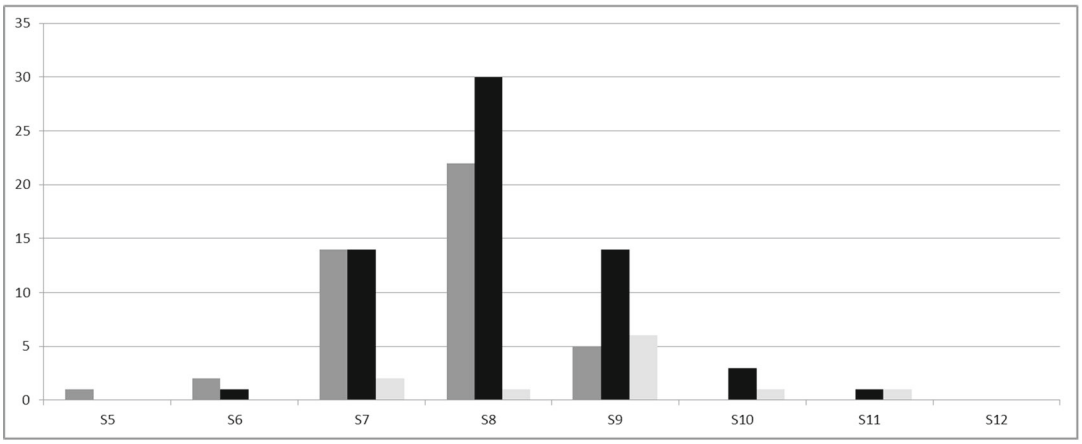
a



Natural conception

Dark grey: Normal; Black: Trisomy; Lightgrey: Monosomy.

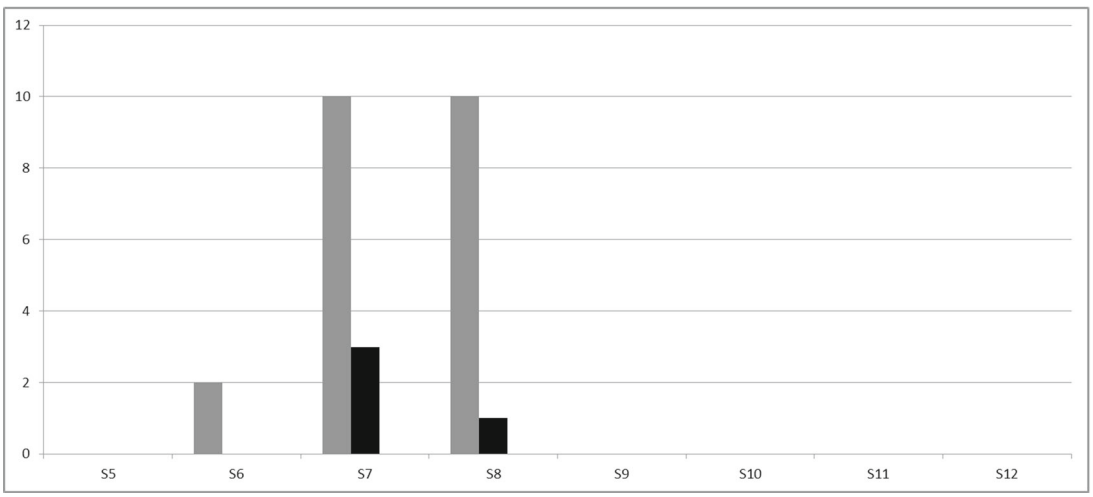
b



ART using the patient's own oocyte.

Dark grey: Normal; Black: Trisomy; Lightgrey: Monosomy.

c



ART using a donated oocyte.

Dark grey: Normal; Black: Trisomy; Lightgrey: Monosomy.

◀ **Fig. 4** Results according to the gestational age. **a**: Natural conception. *Dark grey*: Normal; *Black*: Trisomy; *Light grey*: Monosomy. **b**: ART using the patient's own oocyte. *Dark grey*: Normal; *Black*: Trisomy; *Light grey*: Monosomy. **c**: ART using a donated oocyte. *Dark grey*: Normal; *Black*: Trisomy; *Light grey*: Monosomy. Miscarriages with abnormal results are mainly concentrated at around weeks 7–8 and 9 weeks gestation, with the highest incidence occurring in week 8 of gestation. There are no differences in the type of gestation origin and gestational age at which spontaneous abortions with chromosomal abnormalities appear

age. Regarding the male contribution, an increase in sperm chromosomal abnormalities due to meiotic impairment has been described in male factor (MF) infertility [38, 39]. Moreover a low sperm count also increases the percentage of chromosomally abnormal sperm and therefore the risk of producing aneuploid embryos. In our study, 75 % of the POC samples had an abnormal result if the sperm concentration was less than 5 million/mL whereas and 51 % of the samples had an abnormal result if the concentration was greater than 5 million/mL. Although these results show a trend, they are not statistically significant which we suspect is due to a sample size limitation for our population sample.

More recently other molecular techniques have been applied to the study of POC samples. One of them is array CGH analysis which has been used in several studies to analyze spontaneous abortions [40]. This technology, as well as KaryoLite™ BoBs™, has several advantages compared to traditional cytogenetics because it does not require cell culture and thus avoids the possibilities of culture failure, culture contamination, maternal cell overgrowth, or selection against chromosomally abnormal cells derived from mosaic fetuses [7]. Although array CGH fails to detect triploidies in the majority of cases, it can identify a number of smaller deletions and duplications [7] and previous studies have shown that array CGH with BACs can detect low-grade mosaicism at the lowest threshold currently available, at around 10 % [41–43]. By using 47,XXY reference DNA, the difference between sex chromosome abnormalities, such as X chromosome trisomies and tetrasomies, and normal 46,XX and 46, XY samples is more readily observable [40], which confirms previous results from Ballif et al. [40] Additionally, some triploidies (XXY but not XXX) and some tetraploidies (all XXYY) can also be detected using this technique [7].

In summary, we compared the role of maternal age, sperm quality, and gestational origin as risk factors in abnormal gestations using efficient, high-resolution, KaryoLite™ BoBs™ technology to analyze POC samples.

By applying the insights gained by these types of study couples can be appropriately counseled on different reproductive strategies if aneuploidy is identified in a POC which could help them to overcome the risk of genetic abnormalities subsequent pregnancies. Preimplantation genetic screening (PGS) and chromosomal analysis of the embryos before transfer into the uterus is one such possible strategy to help couples conceive and deliver a healthy full-term baby. PGS with the analysis of a limited number or chromosomes by FISH has also been successfully applied in couples with a previous aneuploidy pregnancy [44].

Compliance with ethical standards The present study was conducted after the approval from our IRB.

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