Austin Journal of Obstetrics and Gynecology

Open Access

Austin Publishing Group

Research Article

FMR1 CGG Repeat and AGG Interspersion Number in Female Newborns Conceived by Assisted Reproductive Technologies

Elizabeth X Wu and Sai Ma*
Department of Obstetrics and Gynaecology, University of British Columbia, Vancouver, BC, Canada

***Corresponding author:** Sai Ma, PhD,
Department of Obstetrics and Gynaecology University of British Columbia
BC Women's Hospital and Health Centre Room D414B, 4500 Oak Street Vancouver, British Columbia, Canada V6H 3N1
Tel: 1-604-87502591;
Email: msai914@gmail.com

Received: February 13, 2025;
Accepted: March 10, 2025;
Published: March 13, 2025;

Abstract

Objective: High repeat *FMR1* alleles are associated with Fragile X syndrome (FXS), fragile X- associated tremor/ataxia syndrome (FXTAS), and premature ovarian failure (POF). It is possible that infertile individuals are passing on *FMR1* alleles associated with infertility or that repeat expansion could be occurring in subfertile parent's germlines or during *in vitro* culturing. This could put children conceived through assisted reproductive technologies (ART) at a greater risk of developing FXS, POF, and FXTAS. The objective of this study was to assess *FMR1* repeat length in females conceived by intracytoplasmic sperm injection (ICSI) and *in vitro* fertilization (IVF).

Methods: *FMR1* CGG repeat and AGG interspersion number was determined in female newborns conceived by ICSI (n=75) and IVF (n=71) using the Asuragen AmplideX™ *FMR1* PCR Kit. PCR products were sized by capillary electrophoresis.

Results: No differences were found between the frequencies of intermediate and premutation alleles in the ART groups and a previously published group of women from the general population. No premutation or full mutation alleles were detected in ART populations. However, the distribution of CGG repeats was found to be different between the ICSI and general population groups ($P<0.05$).

Conclusions: The significance of these findings is unclear as different ethnic groups have been shown to have differing *FMR1* repeat distributions. Information on ethnic origin was unavailable for our subjects. Our findings suggest that females conceived by ICSI and IVF are not at a greater risk of developing FXS, POF, or FXTAS due to *FMR1* alleles with higher CGG repeat counts and more instability.

Keywords: *FMR1*; CGG repeats; ICSI; IVF; Premature ovarian failure

Introduction

Fragile X syndrome (FXS), the most common cause of inherited mental retardation in males, results from the expansion of a trinucleotide CGG repeat located in the 5' untranslated region (UTR) of the *Fragile X mental retardation 1 (FMR1)* gene to >200 repeats (full mutation) [1]. Six to forty-four CGG repeats is considered normal, 45-54 is intermediate, and 55-200 is the premutation range [2-4]. Seventeen percent of male carriers in their 50s [5] and 12.3% of women carriers [6] of the premutation allele display symptoms of the neurodegenerative movement disorder known as fragile X-associated tremor/ataxia syndrome (FXTAS). Premutation alleles are also found in approximately 5% of all premature ovarian failure (POF) cases, 12% of familial POF cases, 3% of sporadic POF cases, but only in 0.4% of the general population [7]. POF is characterized by amenorrhea, elevated gonadotropins, and estrogen deficiency in women under the age of 40 years [8]. In recent years, intermediate ranges of 41-58 repeats [9] and normal ranges of 35-54 repeats [4] have been associated with POF. It has been observed that repeats below and above the range of 26-34 repeats are associated with a decreased ovarian reserve [10]. It has also been suggested that the risk of trisomic pregnancy may be greater in women with intermediate and premutation alleles because a smaller oocyte pool in these individuals may increase trisomy risk [11].

Alleles in the premutation range are prone to expansion to the full mutation range in subsequent generations through mitosis and meiosis due to the instability of large repeat tracts [3]. Loss of trinucleotide AGG repeat interspersions within the CGG repeat tracts are thought to increase repeat instability {Citation} [12]. Loss of AGG interspersions can occur due to deletions or A to C transversions [13]. One to three AGG interruptions are generally found in normal and intermediate alleles while 0-1 are generally found in premutation alleles [14]. An instability threshold has been identified in which uninterrupted CGG tracts of greater than 34-38 repeats have been observed to result in unstable transmission [13]. A recent study has shown that the identification of AGG interruption numbers can improve risk of prediction for expansion of intermediate and small premutation alleles with 45-69 repeats [15].

Citation: Elizabeth X Wu, Sai Ma. *FMR1* CGG Repeat and AGG Interspersion Number in Female Newborns Conceived by Assisted Reproductive Technologies. Austin J Obstet Gynecol . 2025; 12(1): 1228.

It has been observed that infertile women demonstrate a mild shift towards *FMR1* alleles with higher CGG repeat counts [16]. More often, these women seek infertility treatments, such as ovarian hyperstimulation, intrauterine insemination and *in vitro* fertilization (IVF). *FMR1* repeat expansion in the germlines of subfertile parents could also be occurring. Women utilizing infertility treatments are generally advanced in age. Advanced maternal age has been associated with an increased expansion from a premutation allele to a full mutation allele from mother to offspring [17]. In addition, older women are more prone to having gametes with chromosome abnormalities such as aneuploidy [18,19]. Sperm aneuploidy is common in infertile men [20,21]. Meiotic repair mechanisms in infertile individuals could therefore be deficient [22]. This could result in meiotic replication slippage (looping out) or unequal meiotic crossovers leading to *FMR1* repeat expansion. Some studies have indicated that repeat expansions can occur post-zygotically due to mitotic errors [23]. High rates of mosaicism (~25-50%) have been observed in human preimplantation embryos in IVF [24]. It is possible that in this unnatural environment, embryos are more prone to mitotic errors which could result in repeat expansion by mitotic replication slippage or unequal sister chromatid exchange in the post-zygotic stage. Therefore, there could be an increased risk of female offspring conceived through assisted reproductive technologies (ARTs) inheriting *FMR1* alleles with higher repeats and developing POF, FXTAS, and/or FXS.

It is also possible that females conceived by IVF may have *FMR1* alleles with higher repeats than females conceived by intracytoplasmic sperm injection (ICSI). IVF is mainly used to treat female factor infertility and unexplained infertility. Conversely, ICSI is mainly used to treat male factor infertility or when previous IVF cycles have failed due to low or failed fertilization. Hence, the females of couples which achieve pregnancy via IVF may have undiagnosed POF and be passing on higher repeat *FMR1* alleles that are associated with POF. One study found that the intermediate *FMR1* alleles of POF patients lacked AGG interspersions [9]. Therefore, women with undiagnosed and milder POF may also be passing on *FMR1* alleles with less AGG interspersions and hence increased instability to their children conceived by ARTs.

In this study, we investigated *FMR1* CGG repeat length and AGG interspersion number in female newborns conceived by the ARTs of ICSI and IVF. We also examined *FMR1* genotypes in our ICSI and IVF populations [10].

Materials and Methods Patients and Sample Collection

Patients that conceived through IVF and ICSI were recruited from several IVF centres across Canada between September, 2010 to September, 2014. However, most were from the Lower Mainland of British Columbia. Ethnic information was unavailable for the patients in our study. Little can be assumed about the ethnic backgrounds of our study participants as women were mainly recruited from major Canadian cities, which have a very diverse ethnic population. Therefore, caution was taken when interpreting our findings. Informed consent was obtained for each patient prior to sample collection. A karyotype or comparative genomic hybridization (CGH) analysis of the chromosomes was available for all newborn cases. A total of 146 newborn female umbilical cord blood samples were analyzed in this study. Subjects conceived by ICSI (n=75) included 12 individual females of a set of female twins, 1 individual female of a set of twins where the gender of other twin was unknown, 3 females of a set of mixed gender twins, and 2 females of a set of triplets. Karyotype abnormalities in the ICSI group included one case of 45,XY,-16[2]/46,XX[75] and one case of 46,XX,t(15;18). Subjects conceived by IVF (n=71) included 14 individual females of a set of female twins and 6 females of a set of mixed gender twins. Karyotype abnormalities in the IVF group included one case of 45,XX,-19[4]/46,XX[21]. Blood from the parents of the newborns was not available.

Cord blood samples were collected in EDTA or sodium heparin tubes. The majority of genomic DNA was extracted from cord blood using the QIAamp DNA Mini Kit (Qiagen Inc; Mississauga, Canada). However, some samples were extracted using the traditional salting out method from blood [25] followed by phenol-chloroform extraction to remove the salts and further cleaning using the QIAamp DNA Mini Kit.

Ethical approval for this study was obtained from the University of British Columbia / Children's and Women's Health Centre of British Columbia Research Ethics Board.

FMR1 CGG Repeat Length

The *FMR1* repeat region was amplified by the novel AmplideX™ *FMR1* PCR Kit (Asuragen Inc, Item# 76008; Austin, USA). Forty to one-hundred nanograms of genomic DNA (2µl of DNA at 20-50 ng/µl) was amplified in a master mix containing 11.45 µl of GC-rich AMP Buffer, 1.5 µl of gene-specific *FMR1* forward, reverse (FAM labelled) primers, 0.5 µl of *FMR1* CGG primer, 0.5 µl of diluent and 0.05 µl of GC-rich polymerase mix. The gene-specific primer sequences were as follows: forward 5`-TCAGGCGCTCAGCTCCGTTTCGGTTTCA-3` and reverse 5`-FAM-AAGCGCCATTGGAGCCCCGCACTTCC-3` [26]. The CGG primer was composed of an unlabelled complementary five CGG repeat sequence. PCR conditions were 95°C for 5 min (initial denaturation); 97°C for 35 s, 62°C for 35 s, and 68 °C for 4 min for 10 cycles; 97°C for 35 s, 62 °C for 35 s, and 68°C for 4 min with an additional 20 s per cycle for 20 cycles; and a final extension at 72°C for 10 min. A control mix containing premutated and fully mutated *FMR1* alleles was included in each reaction batch to ensure that premutated and fully mutated alleles were detectable. DNA of carriers of premutated and fully mutated alleles were purchased from the Coriell Institute (Camden, USA; premutated: NA06894; fully mutated; NA07537). The amplified products were stored at -20°C in the dark until analysis by capillary electrophoresis (CE).

Full length PCR products and amplicons were evaluated on an ABI 3130xl Genetic Analyzer (Applied Biosystems; Burlington, Canada) at the Centre for Molecular Medicine and Therapeutics (Vancouver, Canada). Two microlitres of PCR product were mixed with 11 µl of Hi-Di Formamide (Applied Biosystems) and 2 µl of ROX1000 size ladder (Applied Biosystems). The mixture was heat-denatured for 95°C for 2 minutes followed by cooling on ice (protected from light) until transfer to the ABI machine. A 50 cm capillary was used for all injections with an applied voltage of 2.5 kV for 20s with a 40 minute run time at 15 kV on a Pop-7 gel polymer.

PCR products detected by CE were analyzed using GeneMapper

4.0 (Applied Biosystems). The highest point of the gene-specific peak was chosen to represent the size of each allele. Homozygous samples only showed one gene-specific peak and were recorded as having two alleles with the same CGG repeat number. For homozygous samples, it was ensured that no PCR products were detected by CE in the premutation and full mutation range. Normal alleles were defined as <45 CGG repeats, intermediate alleles as 45-54 CGG repeats, premutation alleles as 55-200 CGG repeats, and full mutation alleles as >200 CGG repeats.

Although the increased risk for POF is mainly associated with premutation range (55- 200) alleles, repeat ranges including normal and intermediate alleles such as the repeat ranges of 35-54 repeats [4] and 41-58 repeats [9] have been associated with POF. Therefore, these additional ranges of repeats were assessed in addition to the traditional *FMR1* repeat ranges.

FMR1 AGG Interspersion Number

The method for determining AGG interspersions is as previously described [27]. The CGG repeat primer is specific for CGG repeats and will not bind to AGG sequences commonly found to interrupt the CGG repeat tract. Therefore, dips in the signal intensities correspond to the presence of an intervening AGG. The signal intensity will drop for the equivalent of 5 CGG repeats as each repeat unit on the CGG repeat primer (consists of 5 CGG repeats) mismatches with the AGG sequence [27]. Signal intensities that only drop down half way to baseline indicate that the AGG interspersion is only present on one allele. For the signal intensities to drop to baseline, an AGG must be present at the same position on both alleles [27]. The total number of AGG interspersions (both alleles) was recorded for each sample as it was not always possible to determine the specific number of AGG interspersions on each of the female's alleles.

FMR1 Genotypes

The genotypes are based on a normal range of *FMR1* CGG repeats being between 26-34 repeats [10]. Normal was defined as both alleles within the 26-34 repeat range, het-norm/high as one allele above the range and one within, het-norm/low as one allele below the range and one within, hom-high/low as one allele above and one below the range, hom-high/high as both alleles above the range, and hom-low/low as both alleles below the range [10].

Statistical Analysis

Fisher's exact test was performed to compare the frequencies of intermediate and premutation alleles between the ICSI, IVF, and general populations.

Comparison of the frequency of alleles within the 35-54 and 41-58 repeat ranges was performed using the Chi-Squared test. The Kruskal-Wallis test was used to compare the median allele CGG repeat number and the distribution of CGG repeat numbers between our study groups and a previously published control group [4]. Fisher's exact test was used to compare the frequencies of *FMR1* genotypes in the ICSI and IVF populations. The Mann-Whitney test was performed to compare the median number and distribution of total AGG interspersions between the IVF and ICSI study groups. P values <0.05 were considered statistically significant.

Results

FMR1 CGG Repeat Length

FMR1 allele frequencies for the ICSI and IVF populations as well as a previously published general female population [4] are shown in Table 1. The ICSI and IVF groups were compared with a previously published control group [4] and no differences were found in the frequency of intermediate or premutation alleles (Table 2).

Table 1: *FMR1* allele repeat sizes in female ICSI, IVF, general populations.

CGG Repeat #	ICSI	IVF	General female population[4]
16	1 (0.7%)	0 (0.0%)	2 (0.6%)
17	0 (0.0%)	0 (0.0%)	1 (0.3%)
18	1 (0.7%)	0 (0.0%)	1 (0.3%)
20	12 (8.0%)	10 (7.0%)	31 (9.6%)
21	1 (0.7%)	0 (0.0%)	4 (1.2%)
22	2 (1.3%)	0 (0.0%)	3 (0.9%)
23	5 (3.3%)	7 (4.9%)	30 (9.3%)
24	1 (0.7%)	1 (0.7%)	1 (0.3%)
25	1 (0.7%)	0 (0.0%)	2 (0.6%)
26	2 (1.3%)	1 (0.7%)	1 (0.3%)
27	0 (0.0%)	3 (2.1%)	4 (1.2%)
28	0 (0.0%)	1 (0.7%)	1 (0.3%)
29	28 (18.7%)	39 (27.5%)	71 (22.0%)
30	51 (34.0%)	50 (35.2%)	103 (32.0%)
31	18 (12.0%)	9 (6.3%)	16 (5.0%)
32	8 (5.3%)	2 (1.4%)	20 (6.2%)
33	5 (3.3%)	2 (1.4%)	6 (1.9%)
34	1 (0.7%)	0 (0.0%)	2 (0.6%)
35	0 (0.0%)	0 (0.0%)	0 (0.0%)
36	4 (2.7%)	8 (5.6%)	1 (0.3%)
37	1 (0.7%)	1 (0.7%)	1 (0.3%)
38	0 (0.0%)	0 (0.0%)	6 (1.9%)
39	0 (0.0%)	1 (0.7%)	4 (1.2%)
40	1 (0.7%)	1 (0.7%)	0 (0.0%)
41	1 (0.7%)	1 (0.7%)	2 (0.6%)
42	0 (0.0%)	1 (0.7%)	2 (0.6%)
43	0 (0.0%)	2 (1.4%)	0 (0.0%)
44	2 (1.3%)	1 (0.7%)	2 (0.6%)
45	0 (0.0%)	0 (0.0%)	1 (0.3%)
46	1 (0.7%)	0 (0.0%)	1 (0.3%)
47	2 (1.3%)	0 (0.0%)	1 (0.3%)
48	1 (0.7%)	1 (0.7%)	0 (0.0%)
~70	0 (0.0%)	0 (0.0%)	1 (0.3%)
Full mutation	0 (0.0%)	0 (0.0%)	1 (0.3%)
Total	150	142	322

Table 2: Frequencies of *FMR1* allele repeat ranges and median allele repeat size in ICSI, IVF, and general female populations.

	ICSI	IVF	General female population[4]	*P*-value
Number of samples	75	71	161	
Intermediate alleles (45-54)	4/150 (2.7%)	1/142 (0.7%)	3/322 (0.9%)	ns
Premutation alleles (55-200)	0/150 (0%)	0/142 (0%)	1/322 (0.3%)	ns
Alleles within 35-54 repeat range	13/150 (8.7%)	17/142 (12.0%)	21/322 (6.5%)	ns
Alleles within 41-58 repeat range	7/150 (4.7%)	6/142 (4.2%)	9/322 (2.8%)	ns
Median allele repeat size	30	30	30	ns

ns (non-significant).

Table 3: Distribution and median of total *FMR1* AGG interspersion number in ICSI and IVF populations.

#AGG	ICSI	IVF
0	1 (1.3%)	0 (0.0%)
1	0 (0.0%)	2 (2.8%)
2	8 (10.7%)	9 (12.7%)
3	22 (29.3%)	25 (35.2%)
4	41 (54.7%)	30 (42.3%)
5	3 (4.0%)	5 (7.0%)
6	0 (0.0%)	0 (0.0%)
Total	75	71
Median #AGG	4	3

This control group was chosen for comparison to the groups in our study because the women were representative of the general population of a similar geographic region as our cases [4]. No premutation (55-200 repeats) or full mutation (>200 repeats) alleles were detected in the ICSI and IVF groups. Furthermore, no differences were found between the ICSI, IVF, and the previously published control group [4] in the frequency of alleles within the 35-54 or 41-58 repeat ranges (Table 2). The median allele repeat size of the ICSI, IVF, and the previously published control [4] populations were similar (Table 2). A weak significance was observed between the ICSI, IVF, and general population [4] groups in the distribution of CGG repeats (P=0.0456, Kruskal-Wallis test). A Dunn's Multiple Comparison Test revealed that the difference in CGG repeat distribution was between the ICSI and general population [4] groups (P<0.05).

FMR1 AGG Interspersion Number

The distribution of total AGG interspersion number and the median number of AGG interspersions did not differ between the ICSI and IVF populations (Table 3). One ICSI sample found to have no AGG interspersions had both *FMR1* alleles within the normal range (24 and 30 repeats).

FMR1 Genotypes

The frequencies of *FMR1* genotypes [10] classified by abnormal alleles being above or below the range of 26-34 repeat were similar between the ICSI and IVF populations (Table 4).

Table 4: *FMR1* genotypes in ICSI and IVF populations.

	Norm	het- norm/ high	het- norm/ low	hom- high/ low	hom- low/ low	hom- high/ high
ICSI	45 (60.0%)	7 (9.3%)	16 (21.3%)	2 (2.7%)	3 (4.0%)	2 (2.7%)
IVF	41 (57.7%)	12 (16.9%)	13 (18.3%)	4 (5.6%)	1 (1.4%)	0 (0.0%)
Total	86	19	29	6	4	2

Discussion

Various sizes of *FMR1* alleles have been associated with POF [4,7,9,28,29]. In this study, we set out to investigate whether females conceived by ICSI and IVF are more at risk of developing POF, possibly due to *FMR1* allele sizes associated with reduced fertility, repeat expansion in the germlines of subfertile parents, and repeat expansion during *in vitro* embryo culturing. In addition, we wanted to investigate whether *FMR1* repeats associated with infertility may be more frequent in the IVF conceived population as female factor infertility is a predominant reason for utilizing the IVF procedure to conceive.

Premutation, intermediate, and even normal alleles have been associated with varying degrees of POF. However, we did not observe any differences between the ICSI, IVF, and general populations in the frequencies of alleles within these ranges, indicating that female infants conceived by ARTs are not inheriting higher repeat *FMR1* alleles than females in the general population. It has also been observed that repeats below and above the range of 26-34 repeats are associated with a decreased ovarian reserve [30]. There were no differences between the IVF and ICSI groups in the frequencies of genotypes based on this range. In addition, the majority of females had normal genotypes, suggesting that females conceived by ARTs are not at a greater risk of a prematurely diminished ovarian reserve based on *FMR1* genotypes. However, by splitting up the study populations into such small sub groups based on the Gleicher et al. [30] classifications, our analysis of *FMR1* genotypes did not allow for statistically sound conclusions. The frequencies of *FMR1* repeat lengths and *FMR1* genotypes were similar between the IVF and ICSI conceived female newborn populations. This suggests that *FMR1* alleles associated with POF are not more prominent in the specific population utilizing IVF versus the specific population utilizing ICSI to conceive.

We observed a significant difference in the distribution of CGG repeats between our ICSI group and the published general female population group [4]. This suggests that females conceived by ICSI or couples that are utilizing ICSI to conceive have a different CGG repeat distribution than individuals in the general population. However, the significance is weak and because there was not a higher prevalence of *FMR1* alleles associated with infertility in either group, the clinical implications of this finding is uncertain. Differences in CGG repeat distribution are possibly due to other dissimilarities between our ICSI population and the general population of Bretherick et al. [4]. Differences in the distribution of CGG repeat sizes have been observed between different ethnic population groups [31]. Therefore, the differences in distribution observed between the ICSI group and general population groups [4] may be due to differences in ethnicity between the groups. Patients from both studies were ascertained from the Canadian population. However, the Canadian population is quite diverse and different ethnic groups may comprise more of one group than the other. Unfortunately, the details of ethnic origin of our subjects and of the general population [4] were not available.

The ability of *FMR1* repeat sizes to denote risk for POF may vary between different ethnicities. One study found that the distribution of *FMR1* alleles between infertile females of different ethnicities were distinctly different [30]. Although Asian women experience poorer IVF outcomes than Caucasian patients, they did not have disproportionally more *FMR1* alleles with increased risk for POF [10,30]. This shows that the ability of *FMR1* repeat length to denote POF risk may be limited to certain ethnic populations. As previously mentioned, ethnic information for the patients in our study and the control group of Bretherick et al. [4] was unavailable. Therefore, any observed differences or absence of differences may be due to variations in the distribution of diverse ethnic groups within the three populations.

Many studies have failed to find an association between CGG repeats in the normal or intermediate range and susceptibility to POF [32-34]. Furthermore, if increased expansions are occurring within

the germlines of subfertile parents or during *in vitro* culturing of ARTs, then there should be higher rates of FXS, FXTAS and POF in children conceived by ARTs. However, this has yet to be observed in the literature either because it has not yet been investigated or the effect is not present. It is also possible that epigenetic factors are controlling *FMR1* gene expression rather than solely CGG repeat number. One study found that variability of *FMR1* expression is found in POF patients independent of CGG triplet repeat number [35]. It has been suggested that there could be multiple transcriptional start sites for *FMR1* due to expansions beyond a normal range [35]. It is also proposed that CpG methylation in the repeat region upstream of *FMR1* and compaction of the chromatin around *FMR1* influence the gene's transcription rate [35].

Unfortunately, the comparison of repeat lengths between parents and their offspring was not possible in this study as parental blood was unavailable. A recent study investigated the transmission of seven dynamic mutations, one of which was *FMR1*, between parents and offspring conceived through IVF, ICSI, and naturally [36]. No significance differences were found in the frequency of unstable *FMR1* transmissions between parents and offspring in the three groups studied [36]. None of the subjects in the IVF (n=72), ICSI (n=67), or natural conception (n=75) groups had *FMR1* repeats outside the normal range (6-52 repeats) [36]. Zheng et al. found no differences between the groups in the sizes of *FMR1* repeats or the rates of expansions or contractions [36]. The findings of this group are similar to ours in that there were no significant differences in the frequency of abnormal repeats between study groups. Our study complements Zheng et al.'s study by also investigating the *FMR1* CGG repeat length in ICSI and IVF conceived infants. Our study further categorizes *FMR1* repeat sizes based on various ranges associated with POF in the literature, rather than only using the classical repeat categories which were developed to classify Fragile X syndrome. In addition, we examined AGG interspersions in the *FMR1* repeat tract which are thought to play a major role in repeat stability.

The distribution of total AGG interspersion number between the ICSI and IVF populations was similar. Almost all the alleles had at least one AGG interspersion, which implies that females conceived by ART do not have *FMR1* alleles with greater instability. In the one ICSI sample that had no AGG interspersions on either allele, the repeat numbers of the alleles were both within the normal range (24 and 30 repeats). In one study, uninterrupted CGG tracts of greater than 34-38 repeats were observed to result in unstable transmission from parent to offspring [37,38]. A recent study examining the expansion of 45-69 repeat alleles concluded that there is no apparent risk of full mutation expansions within a single transmission for alleles with 45-49 repeats and no AGGs. Therefore, it is unlikely that the uninterrupted alleles for the ICSI case are unstable as the uninterrupted *FMR1* repeat regions are below the ranges reported in both these studies.

To our knowledge, this is the first study to investigate *FMR1* CGG repeat length concurrently with AGG interspersion number in the alleles of females conceived by ARTs. We did not observe ICSI and IVF populations to be at a greater risk of POF, FXTAS, or FXS due to *FMR1* alleles with a greater number of repeats compared to females in the general population [4]. In addition, we did not observe a greater frequency of *FMR1* alleles associated with POF in the IVF conceived female newborns compared to ICSI conceived female newborns. However, due to the small sample size of our study, there are limitations on what can be concluded. As this is one of the first studies to investigate *FMR1* repeat length in infants conceived by ARTs, further studies are needed to add to the results of this study in order to generate more substantial conclusions regarding the prevalence of different *FMR1* repeat lengths in our specific populations. It would be ideal to genotype the parents as well as their ART conceived newborns. We acknowledge that the lack of parental *FMR1* genotypes limits our ability to detect the impact of ART on the transmission or expansion of *FMR1* alleles. Further studies are also required to further assess whether there is actually a difference between the ICSI and general populations in the distribution of CGG repeats. If a difference is present, details of the ethnic origins of the patients is needed to determine whether the difference in CGG repeat distribution is due to differences between the population born by ICSI and the general population or due to differences in ethnicities between the two groups.

Acknowledgements

We would like to thank the patients for participating in this study. We further thank the University of British Columbia Centre of Reproductive Health, the Genesis Fertility Clinic, the Pacific Centre for Reproductive Medicine, the Calgary Regional Fertility Clinic, other clinics in Canada, and hospitals across the British Columbia Lower Mainland for their assistance in recruitment and sample collection.

References

1. Fu YH, Kuhl DP, Pizzuti A, Pieretti M, Sutcliffe JS, Richards S, et al. Variation of the CGG repeat at the fragile X site results in genetic instability: Resolution of the sherman paradox. Cell. 1991; 67: 1047-1058.
2. Wheeler A, Raspa M, Hagerman R, Mailick M, Riley C. Implications of the FMR1 Premutation for Children, Adolescents, Adults, and Their Families. PEDIATRICS. 2017; 139: S172-S182.
3. Nolin SL, Glicksman A, Ding X, Ersalesi N, Brown WT, Sherman SL, et al. Fragile X analysis of 1112 prenatal samples from 1991 to 2010. Prenat Diagn. 2011; 31: 925-931.
4. Bretherick KL, Fluker MR, Robinson WP. FMR1 repeat sizes in the gray zone and high end of the normal range are associated with premature ovarian failure. Hum Genet. 2005; 117: 376-382.
5. Jacquemont S, Hagerman RJ, Leehey MA, Hall DA, Levine RA, Brunberg JA, et al. Penetrance of the fragile X-associated tremor/ataxia syndrome in a premutation carrier population. JAMA. 2004; 291: 460-469.
6. Coffey SM, Cook K, Tartaglia N, Tassone F, Nguyen DV, Pan R, et al. Expanded clinical phenotype of women with the FMR1 premutation. Am J Med Genet A. 2008; 146A: 1009-1016.
7. Murray A. Premature ovarian failure and the FMR1 gene. Semin Reprod Med. 2000; 18: 59-66.
8. Nippita TA, Baber RJ. Premature ovarian failure: A review. Climacteric. 2007; 10: 11-22.
9. Bodega B, Bione S, Dalpra L, Toniolo D, Ornaghi F, Vegetti W, et al. Influence of intermediate and uninterrupted FMR1 CGG expansions in premature ovarian failure manifestation. Hum Reprod. 2006; 21: 952-957.
10. Gleicher N, Weghofer A, Lee IH, Barad DH. Association of FMR1 genotypes with *in vitro* fertilization (IVF) outcomes based on ethnicity/race. PLoS One. 2011; 6: e18781.
11. Kline J, Kinney A, Brown S, Levin B, Oppenheimer K, Warburton D. Trisomic pregnancy and intermediate CGG repeat length at the FMR1 locus. Hum Reprod. 2012; 27: 2224-2232.

12. Dombrowski C, Lévesque S, Morel ML, Rouillard P, Morgan K, Rousseau F. Premutation and intermediate-size FMR1 alleles in 10 572 males from the general population: Loss of an AGG interruption is a late event in the generation of fragile X syndrome alleles. Human Molecular Genetics. 2002; 11: 371-378.

13. Eichler EE, Macpherson JN, Murray A, Jacobs PA, Chakravarti A, Nelson DL. Haplotype and interspersion analysis of the FMR1 CGG repeat identifies two different mutational pathways for the origin of the fragile X syndrome. Hum Mol Genet. 1996; 5: 319-330.

14. Yrigollen CM, Tassone F, Durbin-Johnson B, Tassone F. The role of AGG interruptions in the transcription of FMR1 premutation alleles. PLoS One. 2011; 6: e21728.

15. Owens KM, Terhaar C, Zdrodowski J, Johnson LR, Eveleigh D. Refining reproductive risk for FMR1 premutation carriers in the general obstetric population Am J Med Genet A. 2022; 188: 1476-1481.

16. Gleicher N, Weghofer A, Oktay K, Barad DH. Correlation of triple repeats on the FMR1 (fragile X) gene to ovarian reserve: A new infertility test? Acta Obstet Gynecol Scand. 2009; 88: 1024-1030.

17. Ashley-Koch AE, Robinson H, Glicksman AE, Nolin SL, Schwartz CE, Brown WT, et al. Examination of factors associated with instability of the FMR1 CGG repeat. Am J Hum Genet. 1998; 63: 776-785.

18. Hassold T, Hunt P. To err (meiotically) is human: The genesis of human aneuploidy. Nat Rev Genet. 2001; 2: 280-291.

19. Heffner LJ. Advanced maternal age--how old is too old? N Engl J Med. 2004; 351: 1927-1929.

20. Kirkpatrick G, Ferguson KA, Gao H, Tang S, Chow V, Ho Yuen B, Ma S. A comparison of sperm aneuploidy rates between infertile men with normal and abnormal karyotypes. Hum Reprod. 2008; 23: 1679-1683.

21. Ferguson K, Chan Wong E, Chow V, Nigro M, Ma S. Abnormal meiotic recombination in infertile men and its association with sperm aneuploidy. Hum Mol Genet. 2007; 16: 2870-2879.

22. Ren H, Ferguson K, Kirkpatrick G, Vinning T, Chow V, Ma S. Altered crossover distribution and frequency in spermatocytes of infertile men with azoospermia PLoS One. 2016; 11: e0156817.

23. Wohrle D, Hennig I, Vogel W, Steinbach P. Mitotic stability of fragile X mutations in differentiated cells indicates early post-conceptional trinucleotide repeat expansion. Nat Genet. 1993; 4: 140-142.

24. Munne S, Weier HU, Grifo J, Cohen J. Chromosome mosaicism in human embryos. Biol Reprod. 1994; 51: 373-379.

25. S Ma 1, D K Kalousek, C Zouves, B H Yuen, V Gomel, Y S Moon. The chromosome complement of cleaved embryos in human *in vitro* fertilization. J *In Vitro* Fert Embryo Transf. 1990; 7: 16-21.

26. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res. 1988; 16: 1215.

27. Filipovic-Sadic S, Sah S, Chen L, Krosting J, Sekinger E, Zhang W, et al. A novel FMR1 PCR method for the routine detection of low abundance expanded alleles and full mutations in fragile X syndrome. Clin Chem. 2010; 56: 399-408.

28. Chen L, Hadd A, Sah S, Filipovic-Sadic S, Krosting J, Sekinger E, et al. An information-rich CGG repeat primed PCR that detects the full range of fragile X expanded alleles and minimizes the need for southern blot analysis. The Journal of Molecular Diagnostics. 2010; 12: 589-600.

29. Sherman SL. Premature ovarian failure in the fragile X syndrome. Am J Med Genet. 2000; 97: 189-194.

30. Wittenberger MD, Hagerman RJ, Sherman SL, McConkie-Rosell A, Welt CK, Rebar RW, et al. The FMR1 premutation and reproduction. Fertil Steril. 2007; 87: 456-465.

31. Gleicher N, Weghofer A, Barad DH. Effects of race/ethnicity on triple CGG counts in the FMR1 gene in infertile women and egg donors. Reprod Biomed Online. 2010; 20: 485-491.

32. Crawford DC, Acuna JM, Sherman SL. FMR1 and the fragile X syndrome: Human genome epidemiology review. Genet Med. 2001; 3: 359-371.

33. Bennett CE, Conway GS, Macpherson JN, Jacobs PA, Murray A. Intermediate sized CGG repeats are not a common cause of idiopathic premature ovarian failure. Hum Reprod. 2010; 25: 1335-1338.

34. Lledo B, Guerrero J, Ortiz JA, Morales R, Ten J, Llacer J, et al. Intermediate and normal sized CGG repeat on the FMR1 gene does not negatively affect donor ovarian response. Hum Reprod. 2012; 27: 609-614.

35. Voorhuis M, Onland-Moret NC, Fauser BC, Ploos van Amstel HK, van der Schouw YT, Broekmans FJ. The association of CGG repeats in the FMR1 gene and timing of natural menopause. Hum Reprod. 2013; 28: 496-501.

36. Schuettler J, Peng Z, Zimmer J, Sinn P, von Hagens C, Strowitzki T, et al. Variable expression of the fragile X mental retardation 1 (FMR1) gene in patients with premature ovarian failure syndrome is not dependent on number of (CGG)n triplets in exon 1. Hum Reprod. 2011; 26: 1241-1251.

37. Zheng YM, Li L, Zhou LM, Le F, Cai LY, Yu P, et al. Alterations in the frequency of trinucleotide repeat dynamic mutations in offspring conceived through assisted reproductive technology. Hum Reprod. 2013; 28: 2570-2580.

38. Eichler EE, Holden JJ, Popovich BW, Reiss AL, Snow K, Thibodeau SN, et al. Length of uninterrupted CGG repeats determines instability in the FMR1 gene. Nat Genet. 1994; 8: 88-94.