A Comparison of the Frequency of Trisomy 13, 18, and 21 Using Non-Invasive Prenatal Testing According to Diminished vs. Normal Egg Reserve and Age

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Abstract: Background: This study’s aim was to determine whether diminished oocyte reserve (DOR) increases the risk of having a fetus with trisomy 13, 18, or 21 at 10 weeks as evaluated by non-invasive prenatal testing (NIPT) and to evaluate the confounding effect of advanced age. Methods: NIPT was undertaken in all pregnancies conceived through natural treatment or assisted reproductive technology that reached 10 weeks from conception with a viable fetus from one infertility center. Data were stratified according to serum anti-Mullerian hormone (AMH) < 1 ng/mL and ≥ 1 ng/mL. Results: No woman < 39 or with AMH ≥ 1 ng/mL showed trisomy 13, 18, or 21 by NIPT. Only women ≥ age 39 with DOR had one of these trisomies. Conclusions: Hopefully these data, coupled with other factors, e.g., etiology of infertility, age, insurance, or financial circumstances, and personal views of pregnancy termination, will aid patients with DOR when choosing treatment options, including natural conception, IVF-ET, IVF with pre-implantation genetic testing for aneuploidy, or transfer of fertilized donor eggs.

Keywords: diminished ovarian reserve; non-invasive prenatal testing; age; aneuploidy

1. Introduction

The risk of Down syndrome (trisomy 21) is the most common autosomal disorder, which occurs in approximately 1 out of 700 live delivered births. Edward syndrome (trisomy 18) is the second most common autosomal disorder, with an incidence of 1 in 3000 live births. Patau syndrome (trisomy 13) occurs in 1 in 6000 live deliveries [1]. The risk of trisomy 13, 18, and 21 increases with age [2–7]. For example, the risk of trisomy 21 is approximately 8 per 10,000 by age 20 and increases to 116 per 10,000 by age 40. Likewise, trisomy 18 occurs in approximately 2 per 10,000 by age 20 and increases to 30 per 10,000 by age 40. Similarly, trisomy 13 occurs in approximately 1 per 10,000 by age 20 and increases to 14 per 10,000 by age 40 [2–7]. Turner syndrome, or 45, X, is unrelated to maternal age and frequently is related to sperm with a missing sex chromosome [1].

The identification of aneuploidy has changed over the last several decades. During the 1970s and 1980s, women over the age of 35, or of advanced maternal age, underwent amniocentesis or chorionic villus sampling to determine aneuploidy [8]. However, this was an invasive procedure associated with an increased risk of pregnancy loss [9]. During the late 1980s and early 1990s, the concept of double, triple, and quad marker testing was developed. Soon after, in the late 1990s and early 2000s, aneuploidy testing shifted to the combined test, which evaluated serum markers and nuchal translucency thickness [8].

Finally, non-invasive prenatal testing (NIPT) was developed, which is currently the gold standard [1,10]. Non-invasive prenatal testing is a screening test that analyzes cell-free DNA (cfDNA) to screen for fetal aneuploidy, and is the most sensitive and specific screening...
test [1,10]. It should be offered to all pregnant patients regardless of age. Fetal cell-free DNA from placental trophoblasts circulates in the maternal blood stream and can be tested for aneuploidy by maternal blood [1] There is over a 99% detection rate for trisomies 21 and 13, and 98% for trisomy 18 [1]. Advantages of this test include the testing period, which can be as early as 9–10 weeks, through to delivery, and it being non-invasive [1]. Disadvantages include diagnosing maternal aneuploidy, as cell-free DNA can be maternal [1]. For example, a woman with trisomy 21 can give birth to a euploid baby, especially if the male partner is chromosomally normal, but NIPT could suggest Down syndrome by measuring maternal DNA. The false positive rate is 2–4%, which warrants further invasive diagnostic testing, such as amniocentesis or chorionic villus [1].

Until recently, NIPT evaluated trisomy 13, 18, and 21. New enhanced technology has allowed some microdeletions to be reported, which include 22q11 DiGeorge, 5p Cri-du-Chat, 15q Angelman, Prader–Willi, 11q Jacobsen, 8q Langer–Giedion, and 4p Wolf–Hirschhorn [11]. However, a meta-analysis was performed exploring positive-predictive values and sensitivity for these common microdeletions that found they ranged from 3–100% and 20–100%, respectively [12]. The authors suggest that NIPT for microdeletions should be used with caution. The American College of Obstetricians and Gynecologists, the American Society of Genetics, and the European Society for Human Genetics do not recommend NIPT for microdeletions [12].

Females are born with a finite number of eggs and there is continued loss over the years [13]. The younger the female, the more antral follicles are generally present in the early follicular phase [13]. However, egg quality diminishes with age, so that fecundity diminishes with advancing age [14].

There are different methods of determining egg reserve. These include day 3 serum FSH, antral follicle count, and anti-Mullerian hormone [15,16]. AMH is secreted by granulosa cells of pre-antral and antral follicles [17]. AMH inhibits primordial follicular development and follicle-stimulating hormone growth [18]. It is one of the best predictors of ovarian reserve and can be used to predict ovarian response [17,19–21]. FSH is considered the most specific test for functional ovarian reserve [22]. A coinciding estradiol (E2) level is necessary to ensure the FSH is not falsely lowered by an elevated E2. A FSH level greater than 10–12 IU/L is consistent with diminished ovarian reserve (DOR) [22]. Antral follicle count (AFC), the number of visible follicles on ultrasound in both ovaries, is also used as a marker of DOR. AFC fluctuates with every menstrual cycle, however, and is less accurate than AMH or FSH [23,24].

There is no unanimous consensus whether women with DOR have both decreased quantity and quality of oocytes. It is difficult to characterize, as reductions in quantity and quality are part of the normal physiologic process of the aging female [25]. Earlier studies suggested that ovarian reserve decline can be due to a qualitative effect and not just a quantitative effect [14]. In addition, DOR can be a result of a variety of factors, including infection, pollution, and lifestyle factors, which can also affect the implantation process and clinical pregnancy rate [26].

Aneuploidy may be a result of age as a function of time-related damage. There is evidence that the decrease in egg quality is related to aging mitochondria in the oocytes [27]. Maternal age-related aneuploidy may be due to faulty spindle assembly checkpoints, recombination errors, and the loss of centromeric cohesion, which is required for meiotic recombination [15,16]. This may cause nondisjunction of chromosomes leading to aneuploidy because of aging [28]. One theory, known as the limited pool hypothesis, is that aneuploidy is inversely related to the number of oocytes [29]. However, it is still controversial whether aneuploidy is more a result of sister chromatid segregation errors due to the size of the follicular pool or due to oxidative stress over time [30].

The mechanism is not known as to how certain resting primordial follicles become primary follicles, which can then have the potential to become the dominant follicle. To ensure survival of the sperm, one possibility exists that a higher amount of mitochondrial DNA leads to a higher chance of becoming a primary follicle [31]. However, there is
evidence that low mitochondrial activity leads to lower implantation rates even when a chromosomally normal embryo is transferred [32].

One previous study found no live deliveries in embryos transferred in women with DOR with serum FSH on day 3 > 20 mIU/mL on three separate occasions regardless of age [33]. The authors suggested that women with DOR are likely to have embryos with rates of aneuploidy similar to their FSH or AMH peers of advanced reproductive age to explain poor pregnancy rates. However, it is well known that pregnancies are possible not only in younger women with DOR, but also in women of advanced reproductive age [34–37].

Because of the concept held by many physicians who treat infertility that DOR means not only a poor pregnancy rate, but a high miscarriage rate and an increased chance of a baby born with aneuploidy, many reproductive endocrinologists/infertility specialists encourage younger patients with DOR to consider the use of genetic testing if they want to use their own eggs, and some suggest they only consider donor eggs from younger women with a normal oocyte reserve (NOR). Often, the infertility specialist advises the patient to grow the embryo to day 5, biopsy the trophectoderm, send the biopsy to genetic labs in order to test for euploidy, and then proceed with a subsequent frozen–thawed blastocyst transfer only if the blastocyst is euploid. An embryo biopsy may lessen the chance that a less hearty embryo can withstand biopsying and the freeze–thaw process. Nevertheless, some couples who, due to religious or ethical considerations, would not terminate a live pregnancy growing in a woman’s body might opt to perform preimplantation genetic testing for aneuploidy (PGT-A) to avoid a baby with trisomy 13, 18, or 21.

There are data suggesting that previous studies suggesting poor live delivered pregnancy rates, even in younger women with DOR, may be related to the high dosage of FSH used [15,16,38]. Nevertheless, some studies have shown that high dosages of FSH make no difference in ongoing pregnancy rates or clinical pregnancy rates [39–41]. Some studies support the concept that DOR by itself does not increase the rate of aneuploidy, nor does the use of high-dosage FSH stimulation [42–44]. However, other studies have shown that patients may require a higher dose of gonadotropins due to DOR, and that high-dosage FSH might not be linked to aneuploidy, or could be linked in combination with several other factors [45–48]. There are studies suggesting that advancing age has a greater negative impact on live delivered pregnancy rates than DOR does [15,16,49].

The present study evaluated NIPT in women with DOR at 10 weeks that delivered a live baby to determine if younger women are more prone to trisomy 13, 18, or 21.

2. Materials and Methods

This was a retrospective study during a two-year period that included women of all ages who, according to serum AMH (≥1 ng/mL versus < 1.0 ng/mL), had NIPT at 10–11 weeks gestational age [50]. It was required that the serum AMH be obtained no earlier than 6 months before achieving pregnancy, and when there was more than one serum AMH within the 6-month window, the AMH used for this study was the one obtained closest to the conception cycle. Pregnancies were either from natural cycles or IVF-ET with the transfer of day 3 embryos. In patients with DOR, there were not enough embryos to allow natural selection of the best embryos by allowing growth to day 5. Some embryos transferred into the uterus on day 3 may result in a live pregnancy, but not develop into a blastocyst in culture. A high percentage of the patient population with normal oocyte reserve previously had IVF elsewhere and experienced poor blastocyst formation, and thus we performed many day 3 embryo transfers, even in patients with normal oocyte reserve. We do, however, perform day 5 embryo transfers on occasion for women with adequate egg reserve, but they were eliminated from this study, to be consistent with the AMH < 1 ng/mL group.

The stimulation protocol for DOR in natural cycles is a form of a mild stimulation protocol known as the FSH receptor upregulation technique, which has a consistent principle, but varies according to serum levels of estradiol, LH, and FSH [15]. This technique has been described in detail according to various hormonal scenarios presented. The IVF stimulation
protocol used a gonadotropin releasing hormone antagonist (cetrorelix or ganirelix) and gonadotropins for follicular advancement. For DOR, oocyte retrieval generally occurred 32 h from the trigger injection of 10,000 IU human chorionic gonadotropin (hCG). For normal oocyte reserve, GNRH ant was also used with a higher dosage of gonadotropins (generally 225–300 IU of FSH). Generally, some LH activity is provided by replacing some of the FSH with human menopausal gonadotropins or low-dosage hCG (10–20 IU). Luteal phase support was provided by a combination of vaginal and oral progesterone. Sometimes intramuscular progesterone was added or substituted.

Protocols for natural cycles also varied according to the degree of DOR and serum FSH, LH, estradiol, and has been described in detail [16].

3. Results

A total of 3 of 193 women who underwent NIPT had a positive trisomy. Two NIPT results were positive for trisomy 18 and one was positive for trisomy 21. All three were in the group that were considered as having DOR based on a serum AMH, as seen in Table 1. Patients with DOR were subdivided into five groups by evaluating the frequency of trisomies 13, 18, and 21 according to the severity of DOR, as seen in Table 1. Interestingly, if one considers the lowest half of the DOR group, i.e., 0 to <0.5 ng/mL, none of the 26 women had a positive NIPT result. The three positive tests were all in the less severe DOR group consisting of 21 patients. Two of the positive tests were in the group with the mildest DOR based on serum AMH.

Table 1. The frequency of trisomy 21, 18, and 13 by non-invasive prenatal testing (NIPT) according to anti-Mullerian hormone < 1 ng/mL.

<table>
<thead>
<tr>
<th>Serum AMH (ng/mL)</th>
<th>Number of Patients</th>
<th>Trisomy 13, 18, or 21</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;0.1</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>0.1 to &lt;0.3</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>0.3 to &lt;0.5</td>
<td>11</td>
<td>0</td>
</tr>
<tr>
<td>0.5 to &lt;0.7</td>
<td>8</td>
<td>1 *</td>
</tr>
<tr>
<td>0.7 to &lt;1.0</td>
<td>13</td>
<td>2 **</td>
</tr>
</tbody>
</table>

* The patient with trisomy 21 was 40.9 years old. ** One patient with trisomy 18 was 39.8 years old and the second patient with trisomy 18 was 39.9 years old.

Table 2 shows the age distribution of patients with DOR. No woman ≤ 38 years old (n = 16) had a positive NIPT result. Two women with a positive NIPT result were very close to age 40 and the other one was close to age 41. Table 3 shows the age distribution of patients with AMH ≥ 1 ng/mL.

Table 2. Age distribution of 47 patients with diminished oocyte reserve (DOR) who had NIPT according to the degree of DOR.

<table>
<thead>
<tr>
<th>AMH Group</th>
<th>Age</th>
<th>No. Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;0.1</td>
<td>&lt;35</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>36–39</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>40–42</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>&gt;43</td>
<td>2</td>
</tr>
<tr>
<td>0.1 to &lt;0.3</td>
<td>35</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>36–39</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>40–42</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>&gt;43</td>
<td>0</td>
</tr>
</tbody>
</table>
Table 2. Cont.

<table>
<thead>
<tr>
<th>AMH Group</th>
<th>Age</th>
<th>No. Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.3 to &lt;0.5</td>
<td>35</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>36–39</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>40–42</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>&gt;43</td>
<td>0</td>
</tr>
<tr>
<td>0.5 to &lt;0.7</td>
<td>35</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>36–39</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>40–42</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>&gt;43</td>
<td>0</td>
</tr>
<tr>
<td>0.7 to &lt;1.0</td>
<td>35</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>36–39</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>40–42</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>&gt;43</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 3. Age distribution of patients with AMH ≥ 1 ng/mL. None had trisomy 13, 18, or 21.

<table>
<thead>
<tr>
<th>Age</th>
<th>Number of Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤35</td>
<td>98</td>
</tr>
<tr>
<td>36–39</td>
<td>44</td>
</tr>
<tr>
<td>40–42</td>
<td>7</td>
</tr>
<tr>
<td>≥43</td>
<td>1</td>
</tr>
</tbody>
</table>

4. Discussion

Our findings suggest that women with DOR (AMH << 1 ng/mL) and aged < 39 were not more prone to deliver babies with trisomy 13, 18, and 21 compared to their age-matched counterparts. These are the three types of aneuploidies that could lead to a live birth, and thus a child with physical or mental ailments. In contrast, women aged > 39 with DOR were shown to have an increased risk of delivering a baby with a trisomy that could lead to a live baby with congenital birth defects. Our study results were consistent with previous findings. Thum et al. concluded that there was no increase in chromosomal abnormality in women aged < 35 with DOR [42]. Similarly, Fouks et al. found that young women, < 40 years old, with DOR did not have increased aneuploidy rates, and had the same live birth rate [43]. Jiang et al. concluded that a low AMH was associated with an increased aneuploidy rate in women with advanced age, but not in women younger than 35 years old [44]. Morin et al. performed a retrospective review of women < 38 years old with DOR, and their findings suggest these women had the same odds of aneuploidy and live birth rate as women with NOR using PGT-A [44]. Of course, there was less chance that a given egg retrieval would yield a chromosomally normal embryo to transfer. However, all these studies used PGT-A [42–44]. Their data suggest aneuploidy was due to a change in the oocyte with aging, and not the oocyte quantity. Using PGT-A to diagnose aneuploidy poses its own challenges, including mosaicism, which are advantages of NIPT.

However, Nasseri et al. found in women with DOR an increase in pregnancy loss, aneuploid pregnancy losses, and aneuploid pregnancies even in young patients [46]. Jaswa et al. also concluded that after adjusting for age, women with DOR based on Bologna criteria were more likely to have aneuploidy vs. women without DOR, but this was also a retrospective study using PGT-A [47]. Shahine et al. suggested that patients with recurrent pregnancy loss (RPL) and DOR compared with RPL and NOR had high aneuploidy rates, specifically more significant in younger patients [48]. These authors suggested that chromosomal screening can decrease miscarriage risk in patients with recurrent pregnancy loss and DOR, as it may result from an increased risk of aneuploid pregnancies.

There are several possibilities to explain the “discrepancy” in these studies. As previously mentioned, high-dosage FSH may possibly be responsible for increased percentages...
of aneuploid embryos determined by PGT in women with DOR versus NOR [15,16,41]. There are no studies published to date that have evaluated the rate of aneuploidy in women with DOR in which the FSH receptor re-uptake technique was used [15,16]. Furthermore, it is possible that women with DOR are more prone to aneuploidy for larger chromosomes, but are not significantly more prone to fetuses with trisomy of the smaller chromosomes, which could lead to live deliveries. This could lead to a somewhat lower chance of conception and increased risk of miscarriage, but not necessarily lead to a live delivery of a baby with organ or brain abnormalities from either trisomy 13, 18, or 21.

Regarding women older than age 39 with AMH < 1 ng/mL, at the time of conception, when deciding to undergo IVF using PGT-A or not, it should be considered that these three fetuses with trisomies might have spontaneously died in the first or second trimester, so the chance of birth with Down syndrome or Edward syndrome may have been much lower than 15.8%.

Thus, based on these data, it does not seem warranted, in general, to recommend that women aged < 39 with DOR undergo preimplantation genetic diagnosis for aneuploidy and its added expense, in view of no evidence of an increased risk of aneuploidy that can lead to a live baby with organ/brain abnormalities. Based on these data, it does not seem absolutely necessary for women aged < 39 with DOR that require treatment with in vitro-fertilization to undergo PGT-A. Women over the age of 39 may be presented with these findings (although emphasizing that this is only one study), and then may consider these data in deciding whether to add PGT-A or not in view of its added expense and the potential to lower the chance of successful implantation due to damage related to freeze–thaw, trophoderm biopsy, and even failure to transfer an embryo diagnosed by PGT-A to have aneuploidy when, in fact, the transfer may have led to a perfectly healthy baby. We generally do not recommend PGT-A, but are open-minded to considering a couple’s views once they understand its benefits and limitations.

It is important to note that this study was underpowered to account for age-matched differences and should be further evaluated with a larger cohort and follow-up of live-birth outcomes. This would require a large multicenter cooperative study to gain sufficient power.

5. Conclusions
The main impact factor for clinical practice based on data accrued by this study is that it provides both treating physicians and patients alike a better understanding as to the likelihood of delivering a baby with an aneuploidy that could cause either physical or mental impairment of that child if conceived despite DOR.

Author Contributions: B.N. wrote most of the manuscript and provided most of the references. N.W. completed chart reviews and gathered data. J.H.C. designed the study and, after B.N., was the second largest contributor to writing the manuscript. C.W., A.D., and M.O. helped N.W. gather data. A.D. was in charge of ensuring that NIPT was undertaken at the right time. M.O., in addition to data collection, typed the manuscript, including many rewrites, before final submission. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: Performing NIPT is a standard of care at the Cooper Institute for Reproductive Hormonal Disorders, which is the REI department for Cooper Medical School of Rowan University and Cooper Hospital. Patients seek our help in either achieving a pregnancy or preventing a miscarriage. We follow all pregnant patients in our clinic for 12 weeks from conception. Part of our standard of care is NIPT at 10 weeks. According to the bylaws of our medical school, the present study, in which there was no randomization, new treatment intervention, or a new diagnostic test, did not require approval from the Institutional Review Board. Nevertheless, this study was approved by the ethics committee of our REI practice.

Informed Consent Statement: All patients, when seen initially, sign a written consent that their data can be used for statistics which could lead to publications. With rare exceptions, patients almost
always agree to this, but we flag anyone refusing to use their data for retrospective studies. None of the patients included in this study refused to allow the use of their data.

**Data Availability Statement:** Data presented in this study are available on request from the corresponding author.

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**Conflicts of Interest:** There are no competing financial interest in relation to data presented herein.

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44. Check, J.H.; Wilson, C. The younger the patients the less adverse effect of diminished oocyte reserve on outcome following in vitro fertilization-embryo transfer as long as the proper ovariain stimulation protocol is used. J. Reprod. Contracep 2013, 24, 221–227.


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