# Genetics – Reproductive

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External PGT-M/PGT-SR and Internal PGT-A Verification of the Agilent OnePGT Solution, powered by Alissa

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OnePGT is a genome-wide next-generation sequencing (NGS) haplimerism-based solution designed to reinforce ranking of IVF embryos. The single workflow solution, consisting of wet-lab reagents and dedicated data analysis software, allows concurrent PGT-M (preimplantation genetic testing of monogenic disorders), PGT-SR (preimplantation genetic testing of structural rearrangements) and PGT-A (preimplantation genetic testing of aneuploidies) hence enabling enhanced genetic profiling.

The OnePGT Workflow

The OnePGT workflow is summarized in Figure 2. Briefly, a single or few cell biopsy from an cleavage or blastocyst stage embryo is subjected to whole genome amplification (Iagen REPLIq 2 hour protocol) and library preparation using proprietary reagents. Only for PGT-M analysis, genomic DNA from the parents and phasing reference(s) are also processed together with the embryo WGA material. The resulting libraries are pooled equimolar for sequencing on Illumina NextSeq 500/550 or HiSeq 2500 Rapid. Demultiplexed sequencing data are then uploaded to the Alissa cloud server and analyzed with the proprietary Alissa OnePGT-PGT-M and/or PGT-A algorithms.

In collaboration with Maastricht UMC+ and KU Leuven, the performance of the OnePGT solution for PGT-M and PGT-SR was assessed by comparing with the site specific reference PGT methods (KUL-SNParray with siCHILD analysis; MUMC- STR-PCR/ArrayCGH/FSH). Due to the reference material available, two different strategies for concordance analysis were required (Figure 3).

A total of 213 embryo biopsy samples were included in this study; 117 samples were kindly donated by KU Leuven and 96 from Maastricht UMC+. All 117 samples from KU Leuven plus 60 from Maastricht UMC+ were evaluated for PGT-M analysis (177 in total) whereas the additional 36 samples from Maastricht were analyzed for PGT-SR. An overview of the results obtained are summarized in Figure 4. Example plots for PGT-M and PGT-SR are provided in Figure 5.

*An additional 14 embryo biopsies were processed but excluded based upon low sample quality including high (≥40%) mitochondrial DNA content (mtDNA), nullisomy of the single gene disorder chromosomes (n=5) and sample contamination (n=1).

A second, internal only study was conducted to demonstrate the sensitivity and specificity of the OnePGT solution for PGT-A. Thirty-six (36) single and forty-eight (48) few cell biopsy samples were manually isolated from Coriell cell lines which collectively display twenty copy number variants of various sizes**. **CNVs tested: 4.1 Mb dup on Chr1, 5 Mb del on Chr15, 7 Mb dup on Chr21, 7.8 Mb del on Chr1, 11 Mb del on Chr17, 12 Mb del on Chr16, 16 Mb del on Chr13, 17 Mb del on Chr14, 22 Mb del on Chr3, 28 Mb dup on Chr3, 30 Mb del on Chr3, Trisomy 21.

The results were subsequently compared with the published Coriell karyotype, summarized in Figure 5. Example PGT-A plots are provided in Figure 7.

Conclusions

Verification of the Agilent OnePGT Solution has demonstrated:

- A combined call rate of 92.5% for PGT-M and PGT-SR.
- An automated call rate of 80.7% for PGT-M and PGT-SR.
- 100% concordance for PGT-M and PGT-SR as compared with two independent reference PGT methods.
- An overall PGT-A sensitivity of 98.5%.
- An overall PGT-A specificity of 94.7%.
- A PGT-A resolution of 20 MB and 5 MB for single and few cells samples respectively.

For Use Only. Not for use in diagnostic procedures.

References

Successful pregnancy and childbirth in a Turner syndrome (45,X) patient with oocyte donation via in vitro fertilization: Case report

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INTRODUCTION

Turner syndrome (TS) is defined as a chromosomal aberration with a total or partial loss of one of the X chromosomes and occurs in 1/2200 to 1/2500 of live born females\cite{1, 2}. Nearly 50% of patients are typical TS cases with monosomy X (45X and mosaic). The remaining 50% of TS cases are caused by the rearrangement of the short arms of the X chromosome. Short stature, web neck, a low or indistinct hairline, low set ears, broad chest with widely spaced nipples, short fingers and toes, and primary ovarian insufficiency are classic features of this syndrome. Primary ovarian insufficiency leads to primary amenorrhea and infertility\cite{3}. In addition, TS patients always accompany with cardiovascular disease, learning disabilities, renal abnormalities, thyroid gland disturbances and psychosocial\cite{4}. Nearly 20% of females with TS, follicles are still present and allow for spontaneous menarche, while spontaneous pregnancies are very rare (2%)\cite{5, 6}. For most women with TS, oocyte donation (OD) with assisted reproductive technology (ART) is the only possible way to get pregnant, despite the limited success rate\cite{7, 8, 9}. Unfortunately, however, up to 60% of these pregnant women with TS may associate with many serious complications\cite{10, 11}. Death in patients with TS was 100-fold in them compare with normal women attempting to pregnant\cite{12}.

CASE REPORT

The patient was diagnosed with TS (45, X) with typical signs of short-stature, web neck and no secondary sexual development at aged 13. The ultrasound showed an infantile uterus and no ovaries. Physical examination did not reveal any disturbances. She underwent human growth hormone therapy for six months since being diagnosed. As absence of ovaries and infantile uterus, hormone replacement therapy (HRT) start at age 13, and three months later she achieved menarche. She accepted the web neck surgery successfully. The height, second sexual sign, uterine structure, other organ system, intelligence, learning ability and mental state were normal in follow-up. At age 26, she was married. The couple took infertility counselling when she was 35 years old. The semen analysis of the patient’s husband was normal. The fertilization was accomplished by oocyte donation with her husband’s semen. She was received an in vitro fertilization embryo transfer (IVF-ET) with an oocyte donation and HRT. Examinations of the multiple system did not reveal any disturbances. No obstetrics complications. Fear of the risk of uterine rupture, other complications and fetal death, finally, the cesarean section was performed at the 37th gestational week. A healthy term male baby, weight 3240g, was born. She gave breastfeeding for 9 months. The patient and the baby were normal in the one year’s follow-up.

CONCLUSIONS

In conclusion, this successful pregnancy in TS with oocyte donation via IVF-ET is rare in China, especially without complications. Our case adds to other cases reported, make statistical results more precise when a review conducted. We want to underline that early diagnosis and HRT treatment with TS is essential for their health. HRT, oocyte donation and IVF-ET are critical for their fertility. All pregnancies in women with TS should be followed by a multidisciplinary team.

REFERENCES


ACKNOWLEDGEMENTS

This work was funded by National Key R&D Program of China (2016YFC1000400) and Xinya Research Fund(kx019) of West China Second University Hospital, SCU.
Potential influence of inflammatory factors on premature ovarian failure

Significance: It is widely believed that there is a close relationship between inflammation and idiopathic POF. PAF-AH (Platelet-activating Factor Acetylhydrolase) and its related factors are the main inflammatory factors.

Method: Three chloroacetic acid precipitation method was used to determine the activity of PAF-AH, H-PAF-AH, L-PAF-AH and H-PAF-AH. The difference in activity levels of each factor between POF patients (35 cases) and controls (91 cases) was compared.

Conclusion: Inflammation may be a risk factor for POF.
INTRODUCTION

Endometrial receptivity is defined as a unique event involving factors that make the endometrium accept implantation of the embryo. This certainly plays an important role in the process of human fertilization. The biochemical markers that arise during the implantation window including Integrin αvβ3 and Prokineticin (PROK-1 or EG-VEGF). To evaluate correlation between PROK-1 (EG-VEGF) serum level and Integrin αvβ3 expression to assess endometrial receptivity in women with regular menstrual cycle.

MATERIALS AND METHODS

This study was a cross-sectional study conducted in Division of Reproductive, Endocrinology and Infertility, Department of Obstetrics and Gynecology Faculty of Medicine, Universitas Sumatera Utara, Haji Adam Malik General Hospital and Adenin Adenan Hospital Medan, from February 2017 until June 2017. The subjects were fertile women with regular menstrual cycles. PROK-1 level was observed from serum and Integrin αvβ3 was observed from endometrial tissue.

RESULTS

From 42 patients, we found the characteristics of fertile women were < 35 years of age (90.5%), Body Mass Index (BMI) was normoweight (73.8%), menarche at < 13 years of age was (66.7%), and period duration was 3-7 days (95.2%).

Table 1. Distribution Subject’s Characteristics

<table>
<thead>
<tr>
<th>Subject’s Characteristic</th>
<th>n = 32</th>
</tr>
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<tbody>
<tr>
<td>Age</td>
<td></td>
</tr>
<tr>
<td>20 – 35 years of age</td>
<td>38 (90,5)</td>
</tr>
<tr>
<td>&gt; 35 years of age</td>
<td>4 (9,5)</td>
</tr>
<tr>
<td>Body Mass Indeks (BMI)</td>
<td></td>
</tr>
<tr>
<td>Underweight</td>
<td>7 (16,7)</td>
</tr>
<tr>
<td>Normoweight</td>
<td>31 (73,8)</td>
</tr>
<tr>
<td>Overweight</td>
<td>4 (9,5)</td>
</tr>
<tr>
<td>Age of Menarche, n (%)</td>
<td></td>
</tr>
<tr>
<td>&lt; 13 years of age</td>
<td>28 (66,7)</td>
</tr>
<tr>
<td>≥ 13 years of age</td>
<td>14 (33,3)</td>
</tr>
<tr>
<td>Duration of Period, n (%)</td>
<td></td>
</tr>
<tr>
<td>3 – 7 days</td>
<td>40 (95,2)</td>
</tr>
<tr>
<td>&gt; 7 days</td>
<td>2 (4,8)</td>
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</tbody>
</table>

Table 2. Correlation between Expression of Integrin αvβ3 and PROK-1 Serum Level

<table>
<thead>
<tr>
<th>PROK-1 (EG-VEGF)</th>
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<tbody>
<tr>
<td>Integrin αvβ3</td>
<td>0,002</td>
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</table>

*Spearman Correlation Test

CONCLUSION

There is a significant correlation between PROK-1 (EG-VEGF) serum level and integrin αvβ3 expression in fertile women.

REFERENCES