pMorph GENETIC TESTING

Personalised medicine in fertility

Pharmacogenomics and personalised medicine offer the potential of improved patient care through the identification of inter-patient genetic variability in drug responses. Whilst the 100,000 genomes project has been crucial in considering how genomic medicine can be used in clinical practice, the uptake is not yet widespread. pMorph is now offering a reliable and cost-effective assay for personalised medicine in fertility, to pre-empt a patients' response to gonadotropin treatment as used in controlled ovarian stimulation. Furthermore, the consequences of the FSH receptor N680S genotype are well established and the allelic frequency of the polymorphism being tested are significant across all ethnicities tested to date.

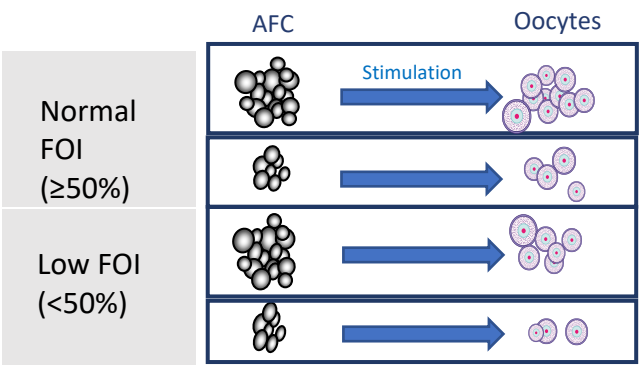
Background info

Whilst the UK live birth rate per embryo transferred has increased to 23% (across all ages) the number of second and third cycles is increasing year on year, indicative of a live birth outcome requiring multi-cycle treatment[1]. Current NICE clinical guidelines in fertility advocate the use of AMH, AFC and/or FSH to predict the likely response to controlled ovarian stimulation and decide on gonadotropin choice and dose[2]. Whilst ovarian biomarkers are indicative of potential we need to reconsider markers of IVF success. Whilst AMH positively correlates with ovarian reserve[3] it has also been shown to negatively corelate with the Follicle Output RaTe (FORT)[4] and whilst AFC has a predictive value regards oocyte retrieval rate[4] it shows a variable correlation with clinical outcomes[5]. Furthermore, the intra and inter-cycle variability of FSH is well established[6] thereby limiting it's potential in treatment and patient management.

The low prognosis patient and hypo-responders

A poor response to stimulation is a condition in which fewer than four follicles and/or oocytes are developed/obtained following ovarian stimulation, with the intention of obtaining more follicles and oocytes[7]. A hypo-response is a phenomenon that manifests with a discrepancy between the number of pre-ovulatory follicles or oocytes which develop following ovarian stimulation as compared to the number antral follicles available at the start of stimulation[8,9] . This is ascertained using FORT or FOI (Follicle Oocyte Index) respectively[8,9]. A diagrammatic representation of FOI is shown below in figure 1.

Figure 1 – Follicle Oocyte Index (FOI)



AFC; bilateral antral follicle count at baseline (3-8mm) Oocytes collected during egg collection

Hypo-responders: Prevalence and aetiology

The Prevalence of poor prognosis patients varies between countries and clinics studied but evidence suggests that upto 55% patients within a fertility setting are considered low prognosis with upto 80% of these due to a hypo-response to controlled ovarian stimulation[10]. Furthermore, a hypo-response is a condition not identified by any current testing methodologies in the UK. Evidence suggests a hypo-response to controlled ovarian stimulation may be due to a gonadotropin or gonadotropin receptor polymorphism[11,12].

The FSHR N680S polymorphism

The FSH receptor polymorphism, and specifically the FSH receptor N680S polymorphism is the most studied and best characterised gonadotropin polymorphism with regards effect on ovarian stimulation[13]. The genetic and protein alterations of the polymorphism are shown below;

Figure 2 – The FSHR N680S polymorphism (rs6166)

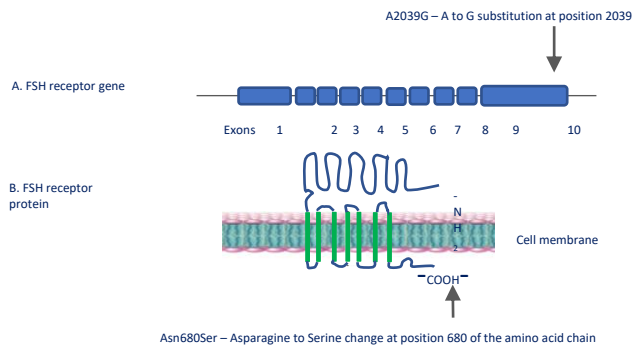


Image adapted from Casarini (2011)

In the example to the left – The first two patients each respond with a Follicle to Oocyte Index (FOI) greater than 50% and an expected response to treatment (irrespective of final oocyte number). The second two patients achieve a FOI <50% which is considered a hypo-response to treatment. Crucially, a hypo-response differs from a poor response and the former is aligned to individual patient potential and baseline AFC.

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Clinical manifestations of the FSHR N680S polymorphism

As discussed a hypo-response leads to a sub-optimal oocyte yield following controlled ovarian stimulation. Consequences of a hypo-response have also been shown to cause diminished clinical outcomes including decreased implantation rates, clinical pregnancy rates and ongoing pregnancy rates when compared to normo-responder patients (as defined by a FORT ≥58%)[14]. A systematic review and meta-analysis has specifically shown the FSH receptor polymorphism (rs6166) to be associated with responsiveness to controlled ovarian stimulation treatment[15]. Women homozygous for GG (at the FSHR locus shown in Figure 2 above) are less responsive to stimulation, produce fewer oocytes and show a reduced gonadotropin to oocyte ration than AA homozygous counterparts[14]. Indeed, *In Vitro* studies in human granulosa cells showed that GG carriers have increased resistance to FSH than do AA carriers[15,16]. The number of FSH ampoules required to achieve ovulation induction and oocyte retrieval was also significantly different (P=<0.05) between women with different FSHR genotypes, with the group homozygous for GG requiring an increased gonadotropin dose compared to the heterozygous (A/G) and homozygous AA groups[17]. Ovarian insensitivity identified in women homozygous for GG cannot be overcome by increasing the daily FSH dose alone[18]. Furthermore, studies have shown reduced oestradiol levels on the day of hCG trigger (following stimulation) in carriers of GG compared to those either homozygous for AA or heterozygotes (A/G), with a linear relationship between the three genotypes and outcome[18,19].

Genotype distibutions of the FSHR N680S polymorphism

Genotype distributions of the FSHR N680S polymorphism are dependent on ethnicity. The frequency of variant giving rise to ovarian sensitivity (GG) across ethnicities is shown below[20]

Ethnic Group	FSHR genotype (%)		
	A/A	A/G	G/G
Caucasian	29.6	48.9	21.5
Asian	50.7	38.8	10.4
Northern Indian	27.3	51.5	21.2
African-American	33.8	47.7	18.5
Mediterranean	26.3	51.4	22.3

Table adapted from Kujiper *et al* 2010

Allelic frequencies of the FSHR polymorphism were identified as 56% Adenine (A) and 44% Guanine (G) at position 2039 of the FSHR gene[21]. This outcome aligns with previously reported outcomes and FSHR genotype was shown to be in Hardy-Weinberg equilibrium[21].

Rationale for pharmocogenomic testing in fertility

Currently utilised diagnostics do not correlate with a gonadotropin or gonadotropin receptor polymorphism and 26,000 women in the UK alone may experience a hypo-response to ovarian stimulation each year. PCR detection of the FSHR genotype is reliable, reproducible and can support personalised treatment protocols to increase implantation and clinical pregnancy rates following stimulation.

1. The Human Fertilisation and Embryology Authority Fertility treatment 2018: Trends and Figures (2020) https://www.hfea.gov.uk/about-us/publications/research-and-data/fertility-treatment-2018-trends-and-figures/ **2.** National Institute of Clinical Excellence clinical guidelines on fertility problems: assessment and treatment; https://www.nice.org.uk/guidance/cg156 **3.** S.L. Broer, B.W.J. Mol, D. Hendriks, F.J.M. Broekmans (2009) *Fertility Steriity* 91 705-714; **4.** V.K. Genro, M. Grynberg, J.B. Scheffer, I. Roux, R. Frydman, R. Fanchin (2011) *Human Reproduction* 26 (3) 671-677 **5**. E. Tsakos, A. Tolikas, A. Daniilidis, B. Asimakopoulos (2014) *Archives of Gynaecology and Obstetrics* 290 1249-1253; **6.** K. Jayaprakasan, Y. Chan, R. Islam, Z. Haoula, J. Hopkisson, A. Coomarassamy, N. Raine-Fenning (2012) *Fertility Sterility* **98** 657-663 **7**. F. Zegers-Hochschild, G.D. Adamson, S. Dyer, C. Rakowsky, J. de Mouzon, R. Sokol, L. Rienzi, A. Sunde, L. Schidt, I.D. Cooke, J.L. Simpson, S. van der Poel (2017) *Fertility Sterility* **108** (3) 393-406 **8.** C. Alviggi, A. Conforti, S.C. Esteves, R. Vallone, R. Venturella, S. Staino, E. Castaldo, C.Y. Andersen, G. De Placido (2018) *Frontiers in Endocrinology* **9** 589-613 **9.** M. Grynberg and J. Labrosse (2019) *Frontiers in Endocrinology* **10** (246) 1-7 **10.** S. Esteves, H. Yarali, L.N. Vuong, J.F. Carvalho, I.Y. Ozbek, M. Polat, H.L. Le, T.D. Pham, T.M. Ho (2021) *Frontiers in Endocrinology* **12** 1-11 **11.** A. La Marca, G. Sighinolfi, C. Argento, V. Grisendi L. Casarinni, A. Volpe, M Simoni (2013) *Fertility Sterility* 99(4) 970-979 **12.** C. Alviggi, A. Conforti, D. Santi, S.C. Esteves, C.Y. Andersen, P. Humaidan, P. Chiodini, G. de Placido, M. Simoni (2018) *Human Reproduction Update* 24(5) 599-614 **13**. L. Casarini, E. Pignatti, M. Simoni (2011) *Reviews in Endocrinology and Metabolic Disorders 12* 303-321 **14.** V. Gallot, A.L. Berwanger da Silva, V. Genro, M. Grynberg, N. Frydman, R. Fanchin (2011) *Human Reproduction* **27**(4) 1066-1072. **15**. L. Casarini, V. Moriondo, M. Marino, F. Adversi, F. Capodanno, C. Grisolia, A. La Marca, G.B. La Sala, M. Simoni (2014 *in vitro Molecular Cell Endocrinology* **393**: 83-91. **16.** L. Casarini, D. Santi, M.Marino (2015) *Reproduction* **150** R175-R184. **17.** M. Perez Mayorga, J. Gromoll, H.M. Behre, C. Gassner, E. Nieschlag, M. Simoni (2000*) Journal of Clinical Endocrinology and Metabolism* **85** 3365-3369. **18.** H. Behre, R.R. Greb, A. Mempel, B. Sonntag, L. Kiesel, P. Kaltwasser, P. Seliger, F. Ropker, J. Gromoll, E. Nieschlag, M. Simoni (2005) *Pharmacogenomics and Genomics* **15**(7) 451-456. **19.** X. Huang, L. Li, L. Hong, W. Zhou, H. Shi, H. Zhang, Z. Zhang, X. Sun, J. Du (2015) *Clinical Endocrinology* **82** 577-583. **20.** E.A.M Kujiper, M.A. Blankenstein, L.J. Luttikhof, S.J.M. Roek, A. Overbeek, P.G. Hompes, J.W.R. Twisk, C.B. Lambalk (2010) *Reproductive Biomedicine Online* **20** 588-593. **21.** I. Lindgren, H. Nenonen, E. Henic, L. Bungum, A. Prahl, M. Bungum, I. Leijonhufvud, I. Huhtaniemi, C.Y. Andersen, Y.L. Giwercman (2019) *Journal of Assisted Reproductive Genetics* **36** 29-38

If you are interested in utilising this test in your clinic and for your patients, or would like further information please contact info@pmorph.com. More details are also available by visiting the website:

www.pmorph.com