Institution: Oxford Brookes University

Unit of Assessment: 5, Biological Sciences

Title of case study: Anti-Müllerian hormone (AMH): from novel antibodies to routine clinical immunoassay tools in worldwide use

Period when the underpinning research was undertaken: 2000–2011

Details of staff conducting the underpinning research from the submitting unit:

Name(s):	Role(s) (e.g. job title):	Period(s) employed by submitting HEI:
Professor Nigel Groome	Professor of Applied Immunology Emeritus Professor of Applied Immunology	[text removed for publication]

Period when the claimed impact occurred: 1 August 2013 to 31 December 2020

Is this case study continued from a case study submitted in 2014? N

1. Summary of the impact

At Oxford Brookes University (OBU), Groome has developed novel, patented monoclonal antibodies (mAbs) that have significantly increased the sensitivity, reliability and speed of measurement of anti-Müllerian hormone (AMH). This provides an assay to assess women's fertility that does not depend on the menstrual cycle and underpins personalised *in vitro* fertilisation (IVF) treatments. These antibodies are licensed to Beckman Coulter and sub-licensed to [text removed for publication] and others, who have developed and launched clinical diagnostic assays for AMH based on OBU's antibodies. Clinicians and healthcare providers worldwide use these assays to assess ovarian reserve, optimise IVF treatments, and diagnose and monitor ovarian granulosa cell tumours to the benefit of millions of women around the world. Royalty income of over [text removed for publication] between August 2013 and July 2020 was received by OBU, indicative of cumulative sales of over [text removed for publication].

2. Underpinning research

Anti-Müllerian hormone (AMH) is a dimeric glycoprotein made by the granulosa cells of the ovary, where it is pivotal for follicular growth and ovarian function. In women, AMH levels correlate with follicle counts. AMH is accepted globally as a direct serum biomarker of ovarian reserve and, as such, is used clinically to assess and monitor the reproductive potential of women.

Groome's contribution to the research and clinical diagnostic applications of AMH has been through the development of innovative mAbs that underpin the accurate and reliable measurement of AMH, and its detection in serum. In 2000, Professor Axel Themmen, Professor of Experimental Endocrinology and Medical Education of Erasmus University (The Netherlands), one of the leading researchers on AMH at the time, invited Groome to join a European Commission-funded grant called OvAGE (R1). Groome's contribution to this project was to develop new mAbs and assays to measure AMH levels (R2, R3a, R3b). Building upon the project's outcomes, a panel of novel mAbs to human AMH were developed and characterised by Groome in 2004, and a pair selected for development of an immunoassay (R4).

The innovation in Groome's approach for AMH came from his decision to immunise not ordinary mice, but AMH knock-out mice with human AMH, thus eliciting the maximum number of mAbs to all

epitopes of human AMH, rather than a pool limited to only epitopes that varied between human and mouse AMH. Subsequently, a pair of novel and highly sensitive mAbs was produced, F2B12H and F2B7A, which could be used to assay different biological samples (R3a). The first antibody (F2B12H) was used as a capture antibody and the second (F2B7A) was used as a detection antibody (R3a, R3b). Although there were other AMH mAb pairs available, such as the pair used in the AMH assay kit from Immunotech, France, Groome's mAbs were directed to the mature region of the hormone, which confers greater resistance to proteolysis, a property that helps to reduce variability in the detection power of the immune assays (R2, R3a). He used these mAbs to develop an enzyme-linked immunosorbent assay (ELISA) to measure AMH levels in human serum. Details of the mAbs, their method of production and the development of the immunoassay are documented in a European patent granted in 2008 (R3a), a US patent granted in 2011 (R3b) and in a co-authored paper (R4) describing use of the human AMH assay to measure AMH in mice.

Further work demonstrated that the mAbs could be used to measure accurately a woman's ovarian reserve in a variety of clinical conditions (R5, R6). Recognising the potential of mAbs for human clinical applications, the mAbs themselves, the hybridoma cell lines that produce them and the patent applications were licensed to [text removed for publication] in 2005 for commercialisation of a manual AMH immunoassay. In the same year, Beckman Coulter bought [text removed for publication] and OBU's patent applications. The patent applications were progressed and the European and US patents were granted in due course (R3a, R3b). From 2005 to 2010 Beckman Coulter continued to sell the [text removed for publication] AMH manual ELISA and then, as [text removed for publication] was closed, relaunched a modified assay known as the Beckman Coulter Gen II AMH ELISA and an automated assay platform.

3. References to the research

R1. 'Early development of ovarian follicles-determination of the timing of menopause', OVAGE Grant agreement ID: QLK6-CT-2000-00338, 1 Jan 2001 to 31 Dec 2004. Funded under: FP5-LIFE QUALITY, Overall budget: EUR1,399,489. **Groome**, **NP** (Principal investigator at Oxford Brookes University)

R2. Weenen C, Laven JS, Von Bergh AR, Cranfield M, **Groome NP**, Visser JA, Kramer P, Fauser BC, Themmen AP. Anti-Müllerian hormone expression pattern in the human ovary: potential implications for initial and cyclic follicle recruitment. *Molecular Human Reproduction*, 2004, 10(2):77-83. DOI: 10.1093/molehr/gah015

R3. Patents

a) European Patent – EP1886140B1, Immunological assays and antibodies for anti-mullerian hormone, **Groome, NP** et al. Granted 18 September 2010. Licensed to Beckman Coulter. https://patents.google.com/patent/EP1886140B1/ar

b) US Patent – 7897350-B2, Immunological assays and antibodies for anti-mullerian hormone, **Groome, NP** et al. Granted 1 March 2011. Licensed to Beckman Coulter. https://patents.google.com/patent/US7897350B2/en

R4. Kenenaar ME, Meerasahib MF, Kramer P, van de Lang-Born BM, de Jong FH, **Groome NP**, Themmen AP, Visser JA. Serum anti-mullerian hormone levels reflect the size of the primordial follicle pool in mice. Endocrinology. 2006 147(7):3228-34. DOI: 10.1210/en.2005-1588.

R5. Anderson RA, Themmen AP, Al-Qahtani A**, Groome NP**, Cameron DA. The effects of chemotherapy and long-term, gonadotrophin suppression on the ovarian reserve in premenopausal women with breast cancer. *Human Reproduction*, 2006 21(10):2583-92. DOI: 10.1093/humrep/del201

R6. Lutchman SK, Mutturishna S, Stein RC, McGarrigle HH, Patel A, Parikh B, **Groome NP**, Daie MC, Chateerjee R. Predictors of ovarian reserve in young women with breast cancer. *British Journal of Cancer*, 2007 96(12):1808-16. DOI: 10.1038/sj.bjc.6603814

4. Details of the impact

The F2B12H and F2B7A mAbs and assay protocols to measure AMH levels in blood serum have had a significant influence on the reproductive health field. Between 2005 and 2013, manual AMH assays using Groome's AMH mAbs facilitated clinical studies that uncovered potential routine clinical applications of serum AMH levels for the improvements of women's' fertility and reproductive health. The impacts described here relate to these applications and the worldwide use of Groome's mAbs in automated AMH assays (Beckman Coulter Access, [text removed for publication] platforms) since 2014 (S1).

Impact on Clinicians and Healthcare Providers

In 2012, users of the Beckman Coulter Gen II AMH assay reported an issue with unreliable detection of AMH in fresh serum samples, resulting in an apparent increase in AMH levels over time while in storage. Groome, by then an Emeritus Professor at OBU, identified the problem and then worked with Beckman Coulter to rectify it (S2). This enabled Beckman Coulter to relaunch the Beckman Coulter AMH Gen II assay in a modified form in 2012 and launch a fully automated version on the Beckman Coulter Access platform in 2014. The impact of automating Groome's AMH assay has been clearly stated by Professor Richard Fleming of Glasgow Centre for Reproductive Medicine Ltd: "The automated Access AMH will deliver increased sensitivity and precision, providing more reliable results for our patients, with absolute values with which we are familiar" (S3). Today, the automated assay is used routinely by IVF labs worldwide to aid prognosis and planning for couples with fertility problems (S4a, S4b). A survey completed by 796 globally distributed IVF clinics indicated that 60% of the respondent IVF clinics use the AMH assay as a first-line test; 54% reported it as the best test for evaluating ovarian reserve (S4b) and 89% reported that AMH results were extremely relevant or relevant to clinical practice (S4b).

The Beckman Coulter Access and [text removed for publication] automated AMH assays gained US Food and Drug Administration approval for diagnostic use in 2017 and 2018, respectively, allowing doctors to incorporate them into routine clinical practice in almost every country in the world. According to the 2018 survey of the College of American Pathologists (CAP), the main benefits of the automated platforms have been threefold: (i) shorter testing times (e.g. 18 minutes for [text removed for publication] AMH) compared with manual assays (3.5 hours) and traditional assays that measure follicle-stimulating hormone, luteinising hormone and oestrogen on the third day of a woman's menstrual cycle; (ii) better performance than manual assays. Users state that "the [text removed for publication] and Beckman Coulter's automated assay detect extremely low and high (0.03 to 23 ng/mL) and produce very little within-patient variability or lab-to-lab variability when using the same assay" (S5); (iii) accurate measurement of AMH. "AMH testing has been a huge advance in the field of reproductive medicine," said Sara Barton, MD, reproductive endocrinologist at the Colorado Center for Reproductive Medicine in Denver. A major issue with the traditional testing approach for ovarian reserve was the duration of the assays (3 days). "But the problem with using only day-three hormone tests is by the time the FSH is abnormal, the prognosis for patients is compromised. They are often in perimenopausal transition. So while it offers an accurate identification of women with poor prognosis, it's already too late to help some of them." (S5). Karen Maruniak, Colorado Center for Reproductive Medicine stated: "The biggest advantage to us has been the time savings and the ability to run the test daily; before, we had to batch the manual test, which took three-and-a-half hours to run" (S5). Dr Robert Veve, US Speciality Labs, San Diego said: "The older test, with so many hands-on responsibilities, opened the door to errors… it is one of the main reasons why so much lab-to-lab variability was observed using the old assay" (S5). CAP surveys are highly significant because the CAP is a US programme that pools information on the productivity and proficiency of clinical laboratories.

The Beckman Coulter Access AMH Assay was winner of the 2015 Scientists' Choice Award® for the Best New Clinical Laboratory Product of 2014, as an "innovative automated AMH test that is helping to improve fertility assessment and treatment" (S6).

Impact on Fertility and Health

AMH assays based on Groome's mAbs have enabled millions of women across the world to make informed life decisions, such as freezing their eggs to give themselves a better chance of getting pregnant after chemotherapy for cancer (S7a) or later in life: "In addition to having less follicles, my level of AMH—which gives a snapshot of your ovarian reserve—was low… This didn't mean I wasn't a candidate for egg freezing, it just meant I'd have to pump myself up with more drugs to get these eggs to mature…" (S7b).

Fertility issues affect 1 in 10 couples globally. This number has increased steadily over recent decades, mainly because women are choosing to have their first child in their mid-30s or later, when their fertility is declining. AMH assays underpin personalised IVF treatments. Ovarian response to stimulation varies considerably from woman to woman and unexpected extreme responses may produce failure from hypostimulation or serious health problems due to hyperstimulation (hyperstimulation syndrome). These problems are avoided by using a recombinant follicle-stimulating hormone (Rekovelle®) dosing algorithm, which is based on a woman's AMH level and body weight (S8). Rekovelle® is more effective than GONAL-f (follitropin alpha), another fertility medicine, at stimulating the ovaries of women undergoing controlled ovarian stimulation for IVF. A trial showed that around 31% of women (204 out of 665) treated with Rekovelle® became pregnant compared with around 32% of women (209 out of 661) treated with GONAL-f. Implantation rates were also similar: around 35% with Rekovelle® versus around 36% with GONAL-f (S8).

AMH assays are also used in paediatrics to investigate abnormal sexual development in boys (S9a). Pubertal delay and congenital hypogonadotropic hypogonadism (HH) share the same clinical manifestation of delayed sexual maturation in prepubertal boys. Levels of gonadotropin and testosterone are very low in prepubertal boys and therefore have little clinical significance. Hence, AMH measurements are useful in the differential diagnosis of pubertal delay and congenital HH. In boys with congenital HH, AMH concentrations are abnormally low; while in pubertal delay AMH concentrations are within the prepubertal reference interval. Low or undetectable levels of AMH are also typical of disorders such as cryptorchidism, anorchia or functional failure or Klinefelter syndrome (S9a).

In combination with Inhibin B or Inhibin A, AMH assays are also used to monitor ovarian tumour recurrence or progression. Elevated levels of any of these markers can indicate the presence of ovarian cancer (S9a). Other uses include the assessment of menopausal status and the ovarian function of women with polycystic ovarian syndrome (S9b).

Impact on Commerce

Beckman Coulter reported worldwide sales of the manual and automated AMH assays of [text removed for publication] from August 2013 to July 2020, giving OBU royalty income of [text removed for publication]. Three sub-licences covering human health, including the multinational healthcare company [text removed for publication], together with two sub-licences for animal health applications, have led to worldwide sales of [text removed for publication] between August 2013 and July 2020, the latter giving OBU royalty income of [text removed for publication] (S10).

5. Sources to corroborate the impact

S1. Automated AMH assay platforms using Groome's monoclonal antibodies

a) Beckman Coulter Access AMH assay: https://www.beckmancoulter.com/en/products/immunoassay/access-amh (launched in 2014, approved by FDA in 2017)

[text removed for publication]

S2. Modification of AMH assay protocol has produced new assay tools

Faye, S, Groome, N, Masica, R and Kertez, G, 2014, Abstract for Poster P-515 'Investigation and resolution of the effect of an interfering factor in the Beckman Coulter Anti-Müllerian Hormone (AMH) Gen II ELISA assay', page e331, ESHRE meeting, Munich, Germany 29 June to 2 July. Available here

S3. Modified AMH assays are more accurate clinical tools

'Automated Anti-Müllerian Hormone Test Available to Improve Fertility Assessment and Treatment', SelectScience, 11 September 2014 [Product News]

S4. Modified AMH assays are used by fertility clinics worldwide

a) 'Putting Patients at the Centre of Fertility Care', James Coker (Reporter, European Medical Journal), EMG Health, 30 August 2018 [link]

b) Tobler, K.J., Shoham, G., Christianson, M.S. et al. Use of anti-mullerian hormone for testing ovarian reserve: a survey of 796 infertility clinics worldwide. *Journal of Assisted Reproduction and Genetics 32*, 1441-1448 (2015). DOI: 10.1007/s10815-015-0562-7

S5. Satisfaction is high with new automated AMH assays

'Satisfaction high with new automated AMH assays', Valerie Neff Newitt, CAP Today, June 2018 [link]

S6. Scientists' Choice Award® to Beckman Coulter for Best New Clinical Laboratory Product 2014, SelectScience, 30 July 2015 [Editorial Article]

S7. Increasing the chances to get pregnant later in life or after cancer treatment

a) USC Fertility, Egg Freezing for Cancer Patients [link]

b) I'm Not Sure I Want Kids – That's Why I Froze My Eggs', Danielle Page, The Well by Northwell [Women's Health]

S8. New analysis of Rekovelle® data further supports use of AMH to personalise fertility treatment, Ferring Pharmaceuticals, 2 July 2018 [Press Release]

S9. Further clinical uses of AMH – two examples

a) Test ID: AMH1, Antimullerian Hormone, Serum, Mayo Clinic Laboratories [link]

b) Anti-Mullerian Hormone, Lab Tests Online[uk] [link]

S10. Licences and Commercial Impact – Commercial and Knowledge Exchange Director, Research Business Development Office, Oxford Brookes University