Institution: Oxford Brookes University

Unit of Assessment: 5, Biological Sciences

Title of case study: Second-generation Inhibin B biomarker assay: impact on male fertility testing and diagnosis and monitoring remission of ovarian granulosa cell cancer

Period when the underpinning research was undertaken: 2000-2013

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	[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

Professor Groome at Oxford Brookes University (OBU) developed the Inhibin B monoclonal antibody 46A/F and an assay procedure that has contributed to significant improvement in the sensitivity and reproducibility of his first-generation Inhibin B enzyme-linked immunosorbent assay (ELISA). The improved assay, the Gen II Inhibin B ELISA, has had major clinical impact through the development of reliable reference ranges for clinical diagnosis of male and female fertility, and the diagnosis and monitoring of granulosa cell tumours of the ovary, improving the treatment and outcomes of patients worldwide. Royalty income was [text removed for publication] between August 2013 to July 2020, which is indicative of cumulative sales in excess of [text removed for publication] across the world.

2. Underpinning research

This impact case focuses on a second-generation Inhibin B assay developed by Professor Groome at OBU, which has lowered the detection limit, and improved the convenience and reliability, of the first-generation Inhibin B assay. Inhibin B is produced by the Sertoli cells in the testes and granulosa cells in the ovaries, which makes its concentration in body fluids a direct indicator of the functional status of testes and ovaries [R1]. Consequently, assaying Inhibin B levels has paved the way for improved male fertility testing, assessment of ovarian reserve/menopause onset and enhanced screening/monitoring programmes for those at risk of ovarian cancer [R1, R2, R3].

In the 1990s, Groome developed antibodies that recognise Inhibins, a group of hormones that regulate the reproductive system by acting on the pituitary gland and blocking the synthesis and secretion of follicle stimulating hormone (FSH). Groome then developed the first clinical assays for Inhibins using his antibodies. Inhibins are heterodimeric molecules containing an α subunit and either a βA (forming Inhibin A) or βB (forming Inhibin B) subunit. Activins contain two β subunits and can be homodimeric or heterodimeric: two βA subunits make Activin A, two βB subunits make Activin B and a βA subunit attached to a βB subunit makes Activin AB. Activin B is closely related to Inhibin B but has the opposite biological effect of enhancing FSH biosynthesis and secretion.

The first-generation Inhibin B assay developed by Groome was widely commercialised in the late

1990s through Oxford Bio-Innovation (OBI), an OBU spin-out company, acquired by Diagnostic Systems Laboratories (DSL) in 1998. DSL was acquired by Beckman Coulter in 2005. The OBI and DSL assays used monoclonal antibodies C5 and R1 developed at OBU [R1]. They comprised multiple steps, including sample oxidation to allow the C5 antibody to recognise its epitope, with the OBI assay including a sample boiling pretreatment step to remove assay interferences [R4, R5], and had sensitivities that were not quite adequate for routine use on some patient samples (such as those from menopausal women that typically contain low levels of Inhibin B) [R6]. Sensitivity could be increased by prolonging the time the antibodies reacted with the serum sample, but this was difficult in routine clinical use. Critically, the low and variable sensitivities of the assays impeded the establishment of reliable reference ranges, which are essential for accurate diagnosis and patient care.

Groome recognised the need to develop simpler, but more accurate and sensitive, assays for Inhibin B. He and his PhD student Helen Ludlow achieved this by raising a new antibody specific for the Inhibin/Activin βB subunit peptide by immunising mice with *Xenopus* laevis Activins [R6]. The resulting 46A/F antibody does not require processing of the sample with heat or oxidising agents, and its sensitivity is threefold higher than that of the C5/R-based assays [R4]. Thus, 46A/F provides a simpler, more sensitive immunoassay for a wider range of sample types, including samples from patients with decreased ovarian reserve. Additionally, 46A/F, unlike C5, can pair with itself in two-site immunoassays, which has led to the first successful immunoassay for the Activin B heterodimer in clinical research [R5, R6]. OBU filed a patent for the new antibody and its applications in the USA, and this was granted in 2013 [R6]. The US patent was licensed exclusively to Beckman Coulter together with the assay for Inhibin B and the component antibodies 46A/F and R1 for commercial exploitation.

3. References to the research

R1. Evans, LW, **Groome, NP** (2001) Development of immunoassays for Inhibin, Activin and follistatin. In: Muttukrishna, S., Ledger, W. (Eds.), Inhibin, Activin and Follistatin in *Human Reproductive Physiology*. Imperial College Press, London, p. 11. ISBN: 9781860942051

R2. Lutchman Singh K, Muttukrishna S, Stein RC, McGarrigle HH, Patel A, Parikh B, **Groome NP**, Davies MC, Chatterjee R (2007) Predictors of ovarian reserve in young women with breast cancer. *British Journal of Cancer*. 96(12): 1808–1816. DOI: 10.1038/sj.bjc.6603814

R3. Young JM, Henderson S, Souza C, Ludlow H, **Groome N**, McNeilly (2012) Activin B is produced early in antral follicular development and suppresses thecal androgen production. *Reproduction*. 143:637-650. DOI: 10.1530/REP-11-0327

R4. Ludlow H, Muttukrishna S, Hyvönen M, **Groome NP** (2008) Development of a new antibody to the human Inhibin/Activin betaB subunit and its application to improved Inhibin B ELISAs. *Journal of Immunological Methods*. 329(1-2):102-11. DOI: 10.1016/j.jim.2007.09.013.

R5. Ludlow H, Phillips DJ, Myers M, McLachlan RI, Kretser DM, Allan CA, Andreson RA, **Groome NP**, Hyvönen M, Duncan WC (2009) A new 'total' Activin B enzyme-linked immunosorbent assay (ELISA): development and validation for human samples. *Clinical Endocrinology.* 71:867-73. DOI: 10.1111/j.1365-2265.2009.03567.x

R6. **Groome NP,** Ludlow H. Antibody to Inhibin/Activin beta B subunit US patent number 8383351 Granted February 26th 2013. https://patents.google.com/patent/US8383351B2/en

4. Details of the impact

The work of Professor Groome has had both significant commercial and clinical impact. The Inhibin B assay has benefitted millions of women and men around the world affected by fertility problems or granulosa cell malignant ovarian tumours.

Impact on commerce

OBU licensed the 46A/F antibodies and test exclusively to Beckman Coulter, which launched an improved Inhibin B manual assay, the Gen II Inhibin B ELISA, in 2012 [S1]. Worldwide sales reported by Beckman Coulter, in more than 60 countries, totalled [text removed for publication] between August 2013 and July 2020, giving OBU an income of [text removed for publication] [S2].

Impacts on human health

Establishing reference ranges for clinical assays

The increased detection range of the Gen II Inhibin B ELISA has led to the establishment of reliable and updated reference ranges for Inhibin B in plasma. The first-generation Inhibin B assay was of limited use in the assessment of testicular function due to a lack of reliable reference ranges. Although the majority of medical decisions are based on information provided by clinical tests, these by themselves are of little value unless they are supported by relevant information for their interpretation. With the Gen II Inhibin B ELISA, reliable reference ranges have been established for Inhibin B in male body fluids. These reference ranges have been key in improving the diagnosis of a number of male genital conditions, including disorders of spermatogenesis and testicular development [S3].

Male infertility

Infertility is estimated to affect 10-15% of couples. Approximately 40% of all infertility cases can be attributed entirely to male factors. Serum Inhibin B levels were rapidly established as an important, sensitive marker of male fertility. Analysis of serum Inhibin B reflects the relationship between Inhibin B, Sertoli cell function and spermatogenesis. Serum Inhibin B is a better marker of male fertility status than FSH because Inhibin B is produced by the Sertoli cells in the testes. Thus, serum Inhibin B has a positive correlation with sperm count and is used for the evaluation of male infertility as a non-invasive predictor of spermatogenesis. The Gen II Inhibin B ELISA is widely used by the National Health Service (NHS) in the UK, and health providers around the world, to assess male fertility, impacting positively on the well-being and fertility outcomes of millions of men and their families [S4]. The increased sensitivity and accuracy of the assay has led to its use as an indicator of the presence of sperm in testes in men with azoospermia. Azoospermia is defined as the complete absence of sperm in ejaculate and is responsible for 10-15% of all infertility in men. Men with azoospermia who test positive for testicular sperm can recover the sperm through testicular extraction procedures and use it for fertility purposes [S5].

Female fertility: ovarian reserve screening

The amount of Inhibin B measured in serum during the start of a new menstrual cycle is directly related to the number and health of small growing follicles. The Beckman Coulter Gen II Inhibin B ELISA is used by fertility clinics to assess ovarian reserve [S6, S7], which assesses the potential number of eggs a woman has in her ovaries. This information is critically important to establish adequate fertility treatments. The Beckman Coulter Gen II Inhibin ELISA is used as an adjunct to FSH and anti-Müllerian (AMH) hormone testing because the combined measurement of these hormones and Inhibin B can better predict ovarian response and pregnancy outcome than FSH or AMH alone [S6, S7]. Before the introduction and validation of these assays as a measure of ovarian reserve, the only evidential source of guidance for mature women interested in their reproductive futures, for whatever reason, was their age. The use of Inhibin B plus the other

assays has significantly improved the clinical advice women receive about their fertility status and is key in enabling them to make life decisions pertaining to their fertility on solid scientific grounds.

Screening for ovarian cancer

Ovarian cancer remains the seventh most common cancer, and eighth most common cause of death from cancer, in women in the world. Although there has been significant improvement in the visualisation of ovarian tumours (e.g. transvaginal sonography), ovarian cancer remains a substantial challenge because of late diagnosis and high mortality rates. However, the discovery that Inhibin B expression is restricted to ovarian granulosa cells in women has led to Inhibin B levels being established as a useful serum marker of granulosa cell turnover. Today, testing for Inhibin B is key in the diagnosis of granulosa cell tumours and mucinous epithelial ovarian tumours [S8]. Ovarian cancer is classified into 3 types: epithelial (80%); germ cell tumours (10-15%); and stromal sex-cord tumours (5-10%), with granulosa cell tumours representing the majority of stromal sex-cord tumours. Inhibin B levels are increased by approximately 60-fold over the range of reference values in 89–100% of patients with granulosa cell tumours [S8]. Inhibin B is also a serum marker for mucinous epithelial tumours, with 55–60% having increased Inhibin B levels, and has a better performance in muscinous and granulosa ovarian cancer than the tumour marker CA125 [S8]. The major impact of Inhibin B testing on women's treatment and outcomes is illustrated by the following comments from a patient with ovarian cancer: "*In Aug 2010 I received a letter from my consultant advising me that my Inhibin levels had risen from their usual bottom of the scale levels and I went to see him. 2 CT scans Sept and Dec 10 showed nothing but my Inhibin B had climbed to 344. I went into hospital for an exploratory laparoscopy in Jan 11, for the docs to discover a 7cm tumour in my abdomen and several small tumours in the pelvic gutter*" [S9].

Development of a clinical assay for myalgic encephalomyelitis/chronic fatigue syndrome

Professor Groome's active pursuit of new international collaborations with scientists and clinicians has led to the discovery that the 46A/F antibody can be used as a biomarker for myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) [S10]. ME/CFS affects around 0.4% of people worldwide and causes unexplained, persistent, and sometimes disabling, fatigue, with no definitive diagnostic tests or treatments available. However, recent clinical studies that have used the 46A/F antibody to monitor levels of Activin B in patients with ME/CFS have shown that levels of this protein are approximately 2-fold higher in patients with ME/CFS compared with controls. 46A/F is able to detect Activin B because this protein is made of two βB peptides, which are recognised by the 46A/F antibody. These studies offer, for the first time, hope of a reliable and accurate test to diagnose ME/CFS and monitor the effectiveness of treatment [S10].

5. Sources to corroborate the impact

S1. Beckman Coulter Gen II Inhibin B ELISA manual [link]

S2. Confidential commercial information showing the income on Inhibin B, 2013–2020, Commercial and Knowledge Exchange Director, Research Business Development Office, Oxford Brookes University

S3. Examples of the use of Beckman Coulter Gen II Inhibin B ELISA to establish reliable reference ranges for plasma Inhibin B

- Barbotin A-L, Ballot C, Sigala J, Ramdane N, Duhamel A, Marcelli F, Rigot JM, Dewailly D, Pigny P, Mitchell V (2015) The serum Inhibin B concentration and reference ranges in normozoospermic. *European Journal of Endocrinology 172*(6): 669-676. DOI: 10.1530/EJE-14-0932
- Molinaro F, Cerchia E, Garzi A, Severi M, Angotti R, Petraglia, Messina M (2016) Serum levels of Inhibin B in adolescents after varicocelectomy: A long term follow up. *Open Medicine 11*(1):204-206. DOI: 10.1515/med-2016-0039

S4. Examples of clinics and pathology departments using Inhibin B tests for testicular function

- Imperial College Healthcare, Test Directory [link]
- Royal United Hospitals Bath NHS [link]

S5. Examples of clinical studies on the use of Beckman Coulter Gen II Inhibin B ELISA to monitor azoospermia

- Alhalabi M (2016) Predictive value of serum Inhibin B levels as an indicator of the presence of testicular spermatozoa in non-obstructive azoospermia. *Middle East Fertility Society Journal 21*(4): 246-252. DOI: 10.1016/j.mefs.2016.05.001
- Isaksson S, Eberhard J, Ståhl O, Cavallin-Ståhl E, Cohn-Cedermark G, Arver S, Lundberg Giwercman Y, Giwercman A (2014) Inhibin B concentration is predictive for long term azoospermia in men treated for testicular cancer. *Andrology 2*(2): 252-258. DOI: 10.1111/j.2047-2927.2014.00182.x

S6. Examples of articles from health organisations discussing use of Inhibin B assay to assess ovarian reserve in females

- 'The Role of Inhibin B in Fertility Treatments', Nicole Galan, verywellhealth, 17 November 2019 [link]
- Inhibin B Test, Women & Infants [link]

S7. Examples of routine NHS lab use of Beckman Coulter Gen II Inhibin B ELISA for assessment of ovarian reserve

- Blood Test Information, Inhibin B, Royal United Hospitals Bath NHS [link]
- Inhibin B, Tests and investigations, Gloucestershire Hospitals NHS [link]

S8. Examples of routine hospital lab use of Beckman Coulter Gen II Inhibin B ELISA in the UK and US for differentiating ovarian tumours with normal CA125 stromal or mucinous epithelial tumours

- Test ID: INHB Inhibin B, Serum, Mayo Clinic Laboratories (a global reference laboratory that helps health care providers worldwide advance patient care and broaden access to specialised testing) [link]
- Test Directory, Inhibin B, ARUP Laboratories [link]
- Inhibin (B), Clinical Use: Testing for inhibin can be used as an aid in the diagnosis of granulosa cell tumours and mucinous epithelial ovarian tumours. It can also be used as an aid in assessing infertility issues. South Tees Hospitals NHS [link]

S9. Compelling example of a patient describing how Inhibin B assays helped to identify and monitor her cancer, Cancer Research UK, Forum post 'Ovarian granulosa cell tumour' [link]

S10. Activin B, a new tool to diagnose ME/CFS

- https://me-pedia.org/wiki/Diagnostic_biomarker
- 'Could we finally have a definitive biomarker for ME/CFS?', ME Research UK, 9 June 2017 [News]