Institution: Oxford Brookes University

Unit of Assessment: 5 - Biological Sciences

Title of case study: Applications of inhibin A and inhibin B immunoassays and their impact in human medicine

1. Summary of the impact (indicative maximum 100 words)

Pioneering new immunoassays for inhibin A and B, developed by Professor Nigel Groome at Oxford Brookes University, have contributed to a significant improvement in the accuracy of pre-natal screening for Downs Syndrome. Use of inhibin A in the 'Quad' and 'integrated' tests, protected by international patents, is widespread in the US (c.3million screened annually) and was recommended by the NHS in 2010, leading to a significant increase in the use of the assays (c.120,000 annually) in the UK. They are also used in clinical diagnostic and monitoring applications for male and female infertility, abnormalities in sexual development in children and ovarian granulosa tumours. Commercialisation has led to royalty income to Brookes which totalled approximately c.£5million in the period January 2008 to December 2012. Brookes has been earning significant sums from the inhibin assays since 1996.

2. Underpinning research (indicative maximum 500 words)

Building upon earlier commissioned work to develop antibodies to inhibin, funding in 1994 from the MRC (grant no. G9330239/1) enabled the development of monoclonal antibodies, assay development and validation which was carried out at by Nigel Groome in his Brookes lab. At the time, the only methods for measuring inhibin were bioassays which were often inaccurate and inconvenient to use and a radioimmunoassay from Monash University which was unable to distinguish bioactive inhibin A & B from the free alpha subunit. The bioactive endocrine role of inhibin is to inhibit production of Follicle Stimulating Hormone (FSH) by the pituitary gland. Groome's research enabled the development of a series of highly specific and robust immunoassays which enabled for the first time the specific measurement of inhibin A and B in human blood samples. It took several years before the assays could be validated for use on human serum and other fluids and the novelty of the inhibin immunoassays from Brookes was to define the biology of inhibin and then to search for clinical applications. This involved overcoming a number of scientific and technical challenges which a number of other labs had failed to achieve.

In 1994 the new inhibin A assay was shown to be able to define the pattern of inhibin A secretion in the menstrual cycle for the first time ever [1]. In 1996, the inhibin B assay was shown to be applicable of defining the pattern of inhibin B secretion in the menstrual cycle, again, for the first time [2]. In 1996 it was shown for the first time in men a negative correlation between a form of inhibin (inhibin B) and serum FSH levels. This demonstrated for the first time that inhibin B was the bioactive relevant inhibin in male physiology [3]. Up to this point the roles of inhibin A and B in men and women and their patterns of secretion had been a mystery and it was only the provision of the Brookes assays which provided the catalytic tools to advance the field. Groome was proactive in finding clinical research collaborators in numerous countries, providing assay materials, and contributing to project design and interpretation leading to many co-authored papers, and started commercialisation of the assays in 1995 with kits put together at Brookes and sold via Serotec. The assays made a net profit every year since then. Further work funded by the MRC (1997, G9627479/1) and later by the EU (1998, BMH4989574) with the same or related inhibin assays developed the first immunoassays for activin A and B, which are dimers of one of the inhibin subunits.

Inhibin as a marker for Down's Syndrome screening:

Working in collaboration with Dr Euan Wallace at Edinburgh University, it was discovered in 1994 that inhibin A is a very useful pre-natal screening marker for Down's Syndrome (DS). This was confirmed in many subsequent papers [4]. A patent application filed in 1994 was subsequently granted in the US in 1999 and many other countries followed. This has been the major commercial clinical application of inhibin assay.

Inhibin in the male:

Following on from the initial inhibin B assay validation in the male [3], it was subsequently shown that there is often a very good correlation between sperm count and serum inhibin B and therefore inhibin B can be used as a surrogate marker for Sertoli cell function and sperm count in population

studies of infertility in males [6]. Sperm cells are nurtured by the Sertoli cells and inhibin B is an indicator that the Sertoli cells are functioning correctly. If inhibin B levels in a man are undetectable then it is unlikely he is capable of sperm manufacture. Some men with normal inhibin B and sperm production may nevertheless have a blockage preventing sperm appearing in ejaculate.

Inhibin in the female:
A long-recognised defining endocrinological feature of women approaching menopause is an increase in follicular phase serum FSH. Groome's work provided for the first time a hormonal explanation for this. The reason for the rise in FSH in early follicular phase is that the lower inhibin B levels in older women, associated with loss of ovarian reserve, releases the pituitary from negative inhibition [7].

3. References to the research (indicative maximum of six references)

[1] **Groome, NP**; Illingworth, PJ; O'Brian, M *et al*, (1994) *Detection of Dimeric Inhibin throughout the human menstrual cycle by 2-site enzyme immunoassay,* Clinical Endocrinology, Vol. 40 Issue 6, Pages: 717-723 (357 citations on WoS on 20/05/2013)
DOI:10.1111/j.1365-2265.1994.tb02504.x

[2] **Groome NP**, Illingworth PJ, O'Brien M *et al* (1996) *Measurement of dimeric inhibin B throughout the human menstrual cycle*, Journal of Clinical Endocrinology & Metabolism. Vol. 81 issue 4, Pages 1401-5, (646 citations in WoS on 20/05/2013)
DOI: 10.1210/jc.81.4.1401
Submitted to RAE2001, Oxford Brookes University, UoA14-Biological Sciences, RA2, NP Groome, Output 1.

[3] Illingworth, PJ; **Groome, NP**; Byrd, W; *et al*., (1996), *Inhibin-B: A likely candidate for the physiologically important form of inhibin in men,* Journal of Clinical Endocrinology & Metabolism, Vol. 81, Issue 4, Pages: 1321-1325 (257 citations on WoS on 20/05/2013)
DOI:10.1210/jc.81.4.1321

[4] Aitken, D; Wallace, E; Crossley, J; Swanston, I; Van Pareren, Y; Van Maarle, M; **Groome, NP**; Macri, J; Connor, JM, (1996), *Dimeric Inhibin A as a marker for Down's Syndrome in early pregnancy,* New England Journal of Medicine, Vol. 334, no.19, (87 citations in WoS on 20/05/2013)
DOI: 10.1056/NEJM199605093341904

[5] **Groome, NP** and Wallace, E (1999), *Method of genetic testing*: US patent number 5952182 N (with other patents worldwide) Patent for Downs screening.

[6] Anawalt, BD; Bebb, RA; Matsumato, AM; **Groome, NP**; Illingworth, PJ; McNeilly, AS; Bremner, WJ (1996) *Serum inhibin B levels reflect Sertoli cell function in normal men and in men with testicular dysfunction*. Journal of Clinical Endocrinology & Metabolism, Vol. 81, issue 9, pages: 3341–3345 (260 citations on WoS on 20/05/2013)
DOI: 10.1210/jc.81.9.3341

[7] Klein, NA; Illingworth, PJ; **Groome, NP**; *et al*., (1996) *Decreased inhibin B secretion is associated with the monotropic FSH rise in older, ovulatory women: A study of serum and follicular fluid levels of dimeric inhibin A and B in spontaneous menstrual cycles,* Journal of Clinical Endocrinology & Metabolism, Vol. 81, Issue 7, Pages: 2742-2745, (266 citations in WoS on 20/05/2013)
DOI:10.1210/jc.81.7.2742

4. Details of the impact (indicative maximum 750 words)

It is through the development of novel immunoassay tools and a very collaborative style of working that Brookes has made its contribution to the inhibin field. Irrespective of the database searched, Groome will be found to be the most published and most cited author in the inhibin field. Initially antibodies and assays were not available from elsewhere and had a catalytic effect on the whole inhibin field. The patent obtained on the application of inhibin A to DS screening [5] enabled effective commercialisation as a clinical diagnostic tool. Commercialisation was launched in 1998

through an Brookes/Serotec spin-out venture (Oxford Bio-Innovation Ltd), and licenses passed on by acquisitions first to Diagnostic System Laboratories (Houston) and then to Beckman Coulter Inc. Licences for the DS patent and worldwide sales of the Brookes antibodies have been exclusive to Beckman Coulter since 2005. Royalty income generated for Brookes by the sales and licences has increased steadily each year since 2000 [a]. The assays remained unique in the marketplace until 2010.

Impacts on Commerce:
Gross royalty income to Brookes from sales of the inhibin assays amounted to £4,937,278 in the period January 2008 to December 2012. In 2012 the antibodies were sold in 61 countries. Brookes has reinvested some of the income into providing studentships and lab equipment to train the next generation of researchers [a]. At least 15 students gained doctorates in Groome's laboratory many in areas related to inhibin. The bulk of the income (around 90%) derived from sales related to Down's Syndrome screening, but some is from other clinical applications as described below.

Impacts on Health and well-being: adoption of a new diagnostic technology;
Down's Syndrome Screening:
This has become the prime clinical application of the inhibin immunoassays. The assay was incorporated onto Beckman Coulter's automated high throughput clinical assay platform and the patent protected the sales for this application. The assay is either used in the second trimester with three other markers as the 'Quad' test or in combination with first trimester markers as the 'integrated' test.

The greatest use of the inhibin A assay for DS screening has been in the USA. The 2012 College of American Pathologists (CAP) report [b], shows that most respondent clinics use the second trimester Quad test with a significant number using the full integrated test. The total usage of the quad or integrated test in 2011 in the USA was over 3 million women screened.

In the USA, it can easily be deduced that the inclusion of inhibin A in the screening for DS has contributed to a significant cost-saving for healthcare. This is through the increased detection rates of the screening kits over the earlier 'triple' test. Modelled predicted detection rates and false-positive rates show that for a 1:270 cut-off, the use of inhibin A in the Quad test has provided a 5% increase in the detection rate while simultaneously reducing the false-positive rate by 0.8% [c]. This improvement over the triple test was confirmed in prospective studies in the USA [c]. Importantly, for affected pregnancies, the average risk is higher when Inhibin A is incorporated while for unaffected pregnancies the risk is lower [c], i.e. the Quad test provided a stronger indication that invasive testing was indicated for affected pregnancies while providing greater reassurance in unaffected cases. When an average DS birth rate of 1 in 700 is assumed, then with no screening programs in the US with 4 million live births a year there would be over 5,700 DS births a year. It can be estimated that lifetime care costs of a DS child would be circa $900,000 per child. The 5% gain in detection as result of adding inhibin-A therefore translates into a potential saving of over $250 million per year. Given the current trend for women to choose to have children later (where there is a high correlation between increasing maternal age and increased instances of DS), this will only become a greater saving. There are also health implications because the amniocentesis procedure carries a 0.5-0.8% chance of provoking a miscarriage; the greater accuracy of the screening means that fewer women have to take that risk [c].

In the UK, the Quad test became the recommended standard of care for second trimester screening in 2010 and is now offered by all but 3 of over 160 NHS trusts. In the period April 2011 to September 2011, Quad and integrated testing increased and accounted for 72.8% of all second trimester tests compared with a previous cycle rate of 49.7%. This represents an estimated 119,360 annual screening rate using the inhibin A test, out of an annual 545,445 total pregnancies screened [d].

Female infertility investigations:

Inhibin A & B assays are important tools in clinics for a full infertility diagnostic workup in women of all ages, due to the importance of Inhibin A&B in the menstrual cycle and ovarian reserve, as described in section 2 [e-g].

Male infertility investigations and environmental monitoring:
Inhibin B assays are used in clinical investigations into male infertility and inhibin B is the only circulating biomarker correlating significantly with sperm count. It has been used to monitor males who are exposed to potentially harmful environments through their occupations [f,g] and to monitor gonadal toxicity after chemotherapy for testicular cancer.

Ovarian Granulosa Cell Tumour monitoring:
Inhibin A & B gives an early indicator of tumour reoccurrence (up to 1 year ahead of symptoms occurring), meaning that treatment can commence earlier leading to a better prognosis for the patient, and potentially saving on treatment costs [h].

Inhibin antibodies in immunopathology:
The R1 monoclonal antibody to the alpha subunit used in the inhibin A and B assays was found in many research studies also to be a useful tool in routine immunopathology to diagnose metastatic or recurrent ovarian and/or adrenal tumours. It is sold by Serotec and Dako [i] for immune-staining of pathology sections. It can help identify ovarian origin for certain metastatic tumours and is used to classify and diagnose adrenal and ovarian cancers. It is used routinely and widely around the world for this purpose.

Applications in diagnosing abnormal sexual development in children:
As the research shown above indicates, inhibin is integral to the physiological reproductive physiological processes, and in the same way that measured levels can be used as markers for underlying problems in adult males and females, they can also be used in diagnostic work-ups concerning abnormal sexual development in children [e-g].

5. Sources to corroborate the impact (indicative maximum of 10 references)

[a] Information available from Brookes showing the income from royalties on Inhibin A & B, 2008-2013.
[b] College of American Pathologists 2012 FP-A Survey. Copy available on request from Brookes' Research and Business Development Office.
[c] Corroborative statement author 1. Statement from Professor of Genetics and Developmental Biology and Director of Diagnostic Human Genetics Laboratories, University of Connecticut Health Center on pre-natal screening for Downs in America (including references to studies demonstrating the figures given). Copy available on request from Brookes' Research and Business Development Office.
[d] UK Fetal Anomaly Screening Programme report 2011-12; http://fetalanomaly.screening.nhs.uk/getdata.php?id=11702
[e] Beckman flyer on inhibin A in fertility workups; https://www.beckmancoulter.com/wsrportal/bibliography?docname=DS14763A%20Access%20Inhibin%20A%20US%20DATA%20Sheet.pdf
[f] Arup Labs clinical information sheet on inhibin B; http://www.aruplab.com/Testing-Information/resources/TechnicalBulletins/Inhibin%20B.pdf
[g] DSL Laboratories (former licence-holders before purchase by Beckmann) information sheet on inhibin B; http://snhs-plin.barry.edu/Research/InhibinBPoster.pdf
[h] Mayo Clinics' description of diagnostic uses of inhibin B in granulosa cell tumour monitoring; http://www.mayomedicallaboratories.com/test-catalog/Clinical+and+Interpretive/88722
[i] Dako product description; http://www.dako.com/uk/ar38/p118850/prod_products.htm?setCountry=true&purl=ar38/p118850/prod_products.htm