Institution: University of Reading

Unit of Assessment: Unit of Assessment 5: Biological Sciences

Title of case study: Development of an ultra-sensitive immunoassay for Inhibin-A and its utility as a clinical screening marker for Down's syndrome

1. Summary of the impact

Pregnant women and public health service providers have benefitted since 2003 from the development of an ultra-sensitive immunoassay for inhibin-A – a hormone that is produced by the placenta during pregnancy and that is elevated in Down's syndrome pregnancies. The assay, developed by Professor Groome at Oxford Brookes University and Professor Knight at the University of Reading in 1994, was the first test capable of quantifying low levels of inhibin-A in the peripheral blood of humans. Addition of this test to existing antenatal screening tests improved the Down's syndrome detection rate from 59% to 70% and from 67% to 77% when combined with ultrasound imaging. Addition of inhibin-A as the fourth marker measured in the maternal blood serum became known as the quadruple or quad test and was adopted into UK clinical guidelines in 2003. It remains the recommended screening test for women who present themselves in the 2nd trimester. Since 2008 hundreds of thousands of UK women and their healthcare providers have benefitted from the additional information provided by this more accurate screening method, including whether more invasive diagnostic tests are wanted. The quadruple test has been widely adopted in the clinical guidelines in other countries including the US, Canada, and Australia.

2. Underpinning research

Background:

In the mid-1980s Professor Knight began work at the University of Reading on an ovarian hormone called inhibin that negatively controls follicle-stimulating hormone (FSH) release from the pituitary. Inhibin is a protein complex that consists of an alpha subunit and one of two beta subunits (A or B) and is closely related to the hormone activin, which has the opposite biological effect of enhancing FSH biosynthesis and secretion. Knight purified inhibin and demonstrated that antibodies against the hormone could increase ovulation rate (sheep, cattle, chickens) and litter size in sheep. Recognising a need to measure blood concentrations of inhibin and activin in both animals and humans, Knight began work, in collaboration with Professor Nigel Groome at Oxford Brookes University, to develop immunoassays for these hormones.

The 'first generation' of inhibin-A and activin-A assays, developed in the early 1990s, lacked sensitivity and were unable to discriminate between the different molecular forms based on the different beta subunits (inhibins A, B; activins A, B, AB). Moreover, these 'first generation' assays cross reacted freely with free inactive inhibin alpha subunits, which lead to serious overestimations of blood hormone levels. Knight and Groome recognised the need to develop an assay that could simultaneously detect both alpha and beta subunits and they subsequently developed the world's first 'two-site' immunoassays (IRMA, ELISA) for inhibin-A (in 1994) [1, 2], activin-A (in 1996) [3] and activin-AB (in 1997).

Improving assay sensitivity:

In 1994, Knight devised a pre-assay oxidation step that improved the binding ability of the inhibin beta A subunit to Groome's monoclonal antibody [1]**.** This not only solved the major problem of binding-protein interference, it provided a ten-fold increase in the sensitivity of the inhibin-A and activin-A immunoassays. Introduction of this critical step in the immunoassay process enabled the quantification of low concentrations of inhibin-A and activin-A in the peripheral blood of humans [2-5]. These assays are used for basic research on the reproductive system of animals and humans, clinical immunodiagnostics and for applied/translational research on assisted reproduction (e.g. monitoring human IVF treatment cycles)**.**

Measuring inhibin concentrations in pregnancy:

In 1995, Knight and his colleagues reported measurements of inhibin-A concentrations in the peripheral serum during a normal human pregnancy [4]. They found that inhibin-A was present in the peripheral blood (principally in the 31kDa form) throughout gestation and that concentrations were up to 50X greater than the maximum values found during the menstrual cycle. These findings supported the idea that inhibin-A and activin-A were being produced by the placenta during pregnancy and established, for the first time, baseline levels of these hormones throughout a

normal pregnancy.

Linking inhibin-A concentrations with Down's syndrome pregnancies:
Between 1994 and 1996, Knight collaborated with Professor Sir Nicholas Wald (Wolfson Institute of Preventive Medicine, Barts and The London School of Medicine and Dentistry), who is a pioneer in the field of antenatal screening for congenital malformations and screening for Down's syndrome in early pregnancy. Knight was keen to apply the inhibin-A and activin-A assays to archived serum samples collected by Wald's group. These samples were assayed in Knight's lab and in 1996 they reported for the first time that second trimester maternal serum inhibin-A levels are raised in Down's syndrome pregnancies [6]. Adding this fourth marker to the existing three markers tested in the serum improved the Down's syndrome detection rate from 59% to 70% [6]. When ultrasound was used to confirm the estimated gestational age of the foetus, the detection rate improved from 67% (three markers (Triple Test)) to 77% (addition of inhibin-A marker (Quadruple Test)) [6].

Knight's significant improvement in the sensitivity of the inhibin and activin immunoassays, in collaboration with Groome, enabled the assays to be used for humans. His measurement of baseline levels of these hormones in normal pregnancies and subsequent measurement in Down's syndrome pregnancies established this assay as an efficient and potentially cost effective method of improving the detection rate in antenatal screening. This enabled other researchers to develop larger clinical studies, which applied the inhibin-A assay to 46,000 pregnancies from 14 UK hospitals between 1996 and 2001 (see Wald *et al.* (2003) *Lancet* 361:835-836 for review). These clinical studies led to the adoption of the Quadruple Test (or Quad Test) into clinical practice, which forms the basis of this impact case study.

3. References to the research These outputs have been submitted to previous RAEs and have been evaluated as of at least 2* quality.

Outputs:
Citations from *Web of Science*, accessed on 08/07/2013, given in parentheses.

[1] Knight, P.G., Muttukrishna, S. (1994) Measurement of dimeric inhibin using a modified two-site immunoradiometric assay specific for oxidized (Met O) inhibin. *J Endocrinol* 141: 417-425. DOI: 10.1677/joe.0.1410417 (cited 56 times)

[2] Muttukrishna, S., Fowler, P.A., Groome, N., Mitchell, G.G., Robertson, W.R., Knight P.G. (1994) Serum concentrations of dimeric inhibin during the spontaneous human menstrual cycle and after treatment with exogenous gonadotrophin. *Hum Reprod* 9: 1643-1642. <http://www.ncbi.nlm.nih.gov/pubmed/7836513> (cited 118 times)

[3] Knight, P.G., Muttukrishna, S., Groome, N.P. (1996) Development and application of a two-site enzyme immunoassay for the determination of 'total' activin-A concentrations in serum and follicular fluid. *J Endocrinol* 14: 267-279. DOI: 10.1677/joe.0.1480267 (cited 224 times)

[4] Muttukrishna S., George L., Fowler P.F., Groome N.P., Knight P.G. (1995) Measurement of serum concentrations of Inhibin-A (alpha-betaA dimer) during human pregnancy. *Clin Endocrinol* 42: 391-397. DOI: 10.1111/j.1365-2265.1995.tb02648.x (cited 114 times)

[5] Muttukrishna S, Fowler P.A., George L, Groome N.P., Knight P.G. (1996) Changes in peripheral serum levels of total activin A during the human menstrual cycle and pregnancy *J Clin Endocrinol Metab*. 81: 3328-3334. DOI: 10.1210/jc.81.9.3328 (cited 142 times)

[6] Wald, N.J., Densem, J.W., George, L., Muttukrishna, S., Knight, P.G. (1996) Prenatal screening for Down's syndrome using Inhibin-A as a serum marker. *Prenat Diagn* 16: 143-153. DOI: 10.1002/(SICI)1097-0223(199602)16:2<143::AID-PD825>3.0.CO;2-F (cited 160 times)

Peer-reviewed grants:
The following peer-reviewed grants were awarded for work on animal models, which supported the development of the inhibin and activin assays used for humans.

Knight (1991-1994) *Studies on the differential production and autocrine/paracrine actions of inhibin, activin and their subunits in the ruminant ovary,* Agricultural and Food Research Council (AG45/594), £88K.

Knight, Cunningham, Savva, Shepherd, Jeacock (1992-1996) *Cattle Reproduction Program: control of ovulation and the maternal recognition of pregnancy,* MAFF (CSA 1957), £1.4M

Knight (1996-1999) *Intra-ovarian roles of activins, inhibins and follistatin in follicular development in cattle,* BBSRC (SO5760), £155K

4. Details of the impact

Context:

In the UK, pregnant women are offered routine tests and checks as part of their antenatal care, including screening for Down's syndrome. Initial screening methods are non-invasive to the foetus as they simply require a blood sample from the mother. These blood serum tests are used to help evaluate whether there is a low or high chance that the baby has Down's syndrome. The results of the initial screens may prompt more invasive diagnostic tests, including chorionic villus sampling or amniocentesis, which can identify any chromosomal abnormalities, but also carry a 1% chance of aborting the foetus. Therefore, more sensitive non-invasive screening methods are extremely beneficial in reducing the number of invasive tests required, reducing risk to the foetus and providing valuable information for parents and health care providers.

In 2003, the UK National Screening Committee (NSC) advised that the benchmarks for detection of Down's syndrome be revised to a 60% detection rate and <5% false positive rate benchmark by 2004/05. The benchmarks were then further revised to a 75% detection rate and <3% false positive rate by April of 2007 as inclusion of the inhibin serum marker would "achieve even better outcomes" [a]. The quadruple test can predict approximately 75% of Down syndrome cases in women under age 35 and over 80% of Down syndrome cases in women age 35 years and older [Reynolds, T (2010) *Int J Womens Health*, 2: 83–88]. In October 2003, the National Institute for Health and Care Excellence (NICE) recommended the quadruple test (which includes the inhibin-A immunoassay) be used for 2nd trimester testing (14-20 weeks) and that the integrated and serum integrated tests, which both include the inhibin-A immunoassay, be used for testing between 11-14 weeks and 14 to 20 weeks as all of these tests met the recommended benchmarks (NICE Clinical Guideline 6, October 2003).

Adoption in UK clinical guidance:

In 2008, NSC recommended revising these benchmarks again, setting a goal of a 90% detection rate and <2% false positive rate by April 2010. They recommended the quadruple test for 2nd trimester testing as approximately 15% of pregnant women do not seek medical advice until the 2nd trimester [b]. This recommendation was adopted in the clinical guidance provided by NICE in 2008 [c] and remains the "[only] screening strategy that is available for women presenting beyond 14 weeks and 2 days pregnancy" [d].

In 2008, the Scottish Government amended their Pregnancy and Newborn Screening Programmes to also include quadruple testing for all women presenting in the 2nd trimester [e].

Personalised information for women in the 2nd trimester:

Though the NHS is shifting toward a combined test carried out in the 1st trimester, tens of thousands of women in the UK who meet with a health professional later in their pregnancy benefit from the 2nd trimester quadruple test. Approximately 750,000 pregnant women are offered NHS screening every year [g] and around 15% of these women (112,500) will come in for testing during the 2nd trimester [b]. Around 60% of these women will accept the screening [f], which means that approximately 67,500 women in the UK benefit annually from the additional information provided by this 2nd trimester screen; this translates to over 370,000 women in the UK between January 2008 and July 2013.

Adoption internationally:

The inclusion of inhibin-A measures as part of the quadruple test in 2nd trimester Down's syndrome screening has been adopted by other countries. In Canada, the province of British Columbia made the Serum Integrated Prenatal Screen (SIPS) available to all pregnant women (regardless of age), which includes the quadruple test, in February 2009 [g]. In the province of Ontario, nearly 67% of pregnant women chose to undergo maternal multiple marker screening (2009-2010 data) [h], which includes a measure of inhibin levels in the mother's blood.

Use of the quadruple test in the 2nd trimester was incorporated into *The Australian Handbook for General Practitioners* in 2007 [i, pg14] and is still the recommended test.

In the US, the quadruple screen is the most widely available and offered test for women presenting in the 2nd trimester, with 86% of surveyed US obstetricians offering the test [Reynolds, T (2010) *Int J Womens Health*, 2: 83–88].

The quadruple test is also offered by the FetalCare centre of excellence in Saudi Arabia, which was established in 2012 [j].

Pregnant women and public health service providers worldwide have benefited from improved antenatal Down's screening programmes through:

- enabling more informed decisions regarding likely pregnancy outcome, particularly with the demographic trend in the developed world for women to have children at a later age when the risk of having a Down's baby is considerably higher; and informing decisions for further action (such as invasive diagnostic tests or pregnancy termination).

5. Sources to corroborate the impact

[a] NHS Fetal Anomaly Screening Programme (2003) *Model of Best Practice for Providing Down's Syndrome Screening Services*, UK National Screening Committee, Public Health England [available in the online archive] <http://fetalanomaly.screening.nhs.uk/archive>.

[b] NHS Fetal Anomaly Screening Programme (2008) *NHS Fetal Anomaly Screening Programme – Screening for Down's syndrome: UK NSC Policy Recommendations 2007-2010: Model of Best Practice,* UK National Screening Committee, Public Health England <

[c] National Institute for Health and Clinical Excellence (2008) *Antenatal care*, NICE Clinical Guideline 62, Issued March 2008 and last modified June 2010. <http://www.nice.org.uk/nicemedia/live/11947/40115/40115.pdf>

[d] NHS Fetal Anomaly Screening Programme (2010) *Review of the Model of Best Practice 2008: Down's syndrome screening for England,* UK National Screening Committee, Public Health England < http://bit.ly/14L579c>

[e] The Scottish Government (2008) *Changes to the Pregnancy and Newborn Screening Programmes*, Public Health and Wellbeing Directorate, CEL 31, July 2008 <http://www.sehd.scot.nhs.uk/mels/CEL2008_31.pdf>

[f] Donnelly, L. (30 Jan 2010) 'Postcode lottery of Down's syndrome screening revealed', *The Telegraph* <http://www.telegraph.co.uk/health/healthnews/7109276/Postcode-lottery-of-Downs-syndrome-screening-revealed.html>

[g] BC Prenatal Screening (2009) *Guideline: Prenatal Screening for Down Syndrome, Trisomy 18 and Open Neural Tube Defects*, BC Prenatal Genetic Screening Program, Provincial Health Services Authority: Vancouver, BC. <http://bcprenatalscreening.ca/sites/genetic/files/Prenatal_Screening_Guideline.pdf>

[h] Better Outcomes Registry & Network (2011) *Highlights from the BORN Ontario LHIN Regional Reports for 2009-2010*, PPT presentation <http://www.bornontario.ca/_documents/Perinatal%20Health%20Report%202009-2010_Provincial%20Overview%20Presentation.pdf>

[i] Barlow-Stewart, K., Emery, J., Metcalfe, S. (2007) *Genetics in Family Medicine: The Australian Handbook for General Practitioners*, The Australian Government Agency and Biotechnology Australia.

[j] FetalCare (2013) *Advanced prenatal diagnosis,* Our Services [website accessed online 15 Jul 2013] <http://www.fetal-care.com/viewservice.php?id=8>.