Institution: Kent

Unit of Assessment: 5, Biological Sciences

Title of case study: Universal approaches to genetic diagnosis in human and non-human IVF embryos [Short title: ICS2_CHR]

1. Summary of the impact

This case study describes the impact of discoveries by Griffin and Handyside on the universal detection of genetic disease in IVF embryos. The team used basic research to develop practicable new techniques now employed in IVF clinics around the world and culminating in a process named "Karyomapping". The impacts are far-reaching and significant: when applied to families at risk of transmitting genetic disorders the process has resulted in live births of unaffected children. The positive results of the discoveries have extended beyond clinical applications: Adaptations of the technology are now being translated to farm animal breeding regimes to improve meat yields and reduce environmental concerns. Impact also includes new product development and wealth generation, job creation, education, and influence on public policy through HFEA, plus widespread public engagement and communication.

2. Underpinning research

The problem

Diagnosis of genetic disease in IVF embryos (Preimplantation Genetic Diagnosis and Screening; "PGD/S") involves removal of single cells, molecular diagnosis, then selective transfer of genetically normal embryos to the uterus. Pioneered by Handyside and Griffin among others, PGD/S is performed either at the chromosomal level (e.g. for Down syndrome) or single gene (monogenic) level e.g. for cystic fibrosis. Although applied successfully to treat more than 10,000 families this technique has several drawbacks. First, approaches to detect chromosome disorders can only examine a subset of the genome. This became a major talking point in reproductive medicine when studies that suggested that contemporary technology was *worsening* IVF success rates. Second, diagnostic tests need to be targeted to specific diseases and thus either chromosomal *or* a solitary monogenic disorder (but significantly not both) could be diagnosed.

The discovery

To circumvent these problems, the Kent team, established in 2004 when Griffin was appointed in the School of Biosciences, made discoveries in the form of practicable new techniques. This first involved an approach using fluorescent probes for all chromosomes [*see 3.1, 3.2*]; the second applied post-genomic technologies to detect both the presence of all chromosome abnormalities and their origin [*see 3.3*]. The third technique took similar technology a step further so that any monogenic *and* chromosome disorders could be detected simultaneously [*see 3.4*]. The resulting technique was named "Karyomapping."

Ioannou et al [*see 3.2*] developed four banks of six fluorescent colour chromosome probe sets that could successfully detect all 24 chromosomes in a single cell using multi-channel fluorescence microscopy. This was developed in collaboration with Kreatech (www.kreatech.com) for the probe sets, the Bridge Centre (www.thebridgecentre.co.uk) for research material, and Digital Scientific UK (www.dsuk.biz) for advanced microscopy techniques. Because of the upcoming development of newer technologies (such as Karyomapping) the approach did not go to clinical diagnostic use but nonetheless found utility for basic research and screening of IVF embryos post PGD/S for verification purposes [*see 3.2*]. Gabriel et al [*see 3.3*] took a different approach (but with the same aim), using amplified DNA to interrogate whole genome DNA "arrays". This had the benefit of being able to detect chromosome abnormalities and their origin at greater resolution and aided the development of Karyomapping**.**

Karyomapping is a universal approach for detection of any chromosome and/or monogenic disease in a single assay [*see 3.4*]. Karyomapping uses amplified DNA to interrogate whole genome DNA arrays and can detect the origin of subtle chromosome abnormalities. In addition, however, the ability to trace the inheritance pattern of monogenic disorders simultaneously was at the core of the discovery. Validation using cystic fibrosis families led to clinical application (manuscripts in preparation), further validation and subsequent clinical trials (unpublished data).

Wider prospects

Development of PGD/S technology including Karyomapping in humans recently led to further basic research activity in pig and cattle IVF. This will have significant impact on agricultural practices and offers a new route to globally sustainable food production. Specifically, IVF with PGD/S has the potential to improve greatly the transport of animals of superior genetic quality around the world. That is, if genotyped *in vitro* fertilised embryos could be transported to nucleus farms in the place of whole animals, this would save the pig and cattle breeding industries many £millions in costs, and reduce environmental damage. Ongoing basic research involving setting up a pig/cattle IVF laboratory in Kent and adaptations of Karyomapping are being used to diagnose agriculturally beneficial traits. This research has significant funding from industry and government (see 3B below)

References to the research *(*Kent-based authors in **bold***)*

3.1. **Ioannou D,** Meershoek EJ, Ellis M, Thornhill AR, **Griffin DK** (2011). Multicolour interphase cytogenetics: 24 chromosome probes, 6 colours, 4 layers. *Molecular and Cellular Probes* **25**:199-205.

3.2. **Ioannou D, Fonseka KGL,** Meershoek EJ, Thornhill AR, **Abogrein A***,* Ellis M, **Griffin DK** (2012)*.* Twenty four chromosome FISH in human IVF embryos reveals patterns of post-zygotic chromosome segregation and nuclear organization. *Chromosome Research* **20**:447-460.

3.3. **Gabriel AS,** Hassold TJ, Thornhill AR, Affara NA, **Handyside AH, Griffin DK** (2011) An algorithm for determining the origin of trisomy and the positions of chiasmata from SNP genotype data. *Chromosome Research* **19**:155-63.

3.4. **Handyside, AH,** Thornhill, AR, **Harton, GL**, Mariani, B, Shaw, MA, Affara, N, **Griffin DK** (2010). Karyomapping: a novel molecular karyotyping method based on mapping crossovers between parental haplotypes with broad applications for preimplantation genetic diagnosis of inherited disease. *Journal of Medical Genetics* **47**: 651-658.

Footnote: Ioannou (2007-2011), Gabriel (2007-2011), Abogrein (2007-2010), Fonseka (2008-2012), and Harton (2010-2013) were PhD students supervised by Griffin at Kent.

Major grants awarded to Griffin and relating to the research: 2011 to date

2011-2015. Technology Strategy Board (BBSRC) with JSR Genetics and Bridge Centre. *Pig IVF and genetics: A route to global sustainability*, £997k including an industrial contribution of £499k. Total grant from TSB = £497k, to Kent = £441k.

2012-2014. Knowledge Transfer Partnership (BBSRC/TSB) with Cytocell Ltd. *To develop a new product line of non-human fluorescence in-situ (FISH) probes to enable Cytocell to become a market leader in the field*. £194k (£130k from BBSRC/TSB, £64k industrial contribution)

2013-2016 Technology Strategy Board (BBSRC) with Paragon vets and Cogent. *Optimising the delivery of superior genetics through advanced genomic selection of bovine embryos,* £1.12 million. Total grant from TSB = £529k, to Kent = £286k

4. Details of the impact

As detailed below, the Karyomapping technology has already had significant and far-reaching impacts on clinical outcomes, on commerce and enterprise, on job creation and training and on public policy and opinion. Its wider application in animal breeding in the longer term will bring further and significant cost-savings to this industry, an impact that is corroborated by the animal farming industry continuing to invest in this research (*see Section 3*). There will also be significant environmental benefits.

1. Clinical applications for patient benefit: Clinical Karyomapping cases have been performed in

real time leading to successful live births of unaffected children [*see 5.1*]. A full clinical evaluation of Karyomapping was performed in IVF centres in the UK, USA, Saudi Arabia, Netherlands and Australia involving over 200 couples. Analysis revealed complete concordance with Karyomapping and "gold-standard" approaches. Plans for full clinical trials are now in advanced stages involving 50-100 patients, beginning in late 2013 with a view to Karyomapping becoming the leading method of performing PGD/S worldwide [*see 5.2*].

2. New product development and wealth creation [*see 5.3*]**:** Kreatech marketed the discovery of Ioannou et al [*see 3.1/3.2 above*] under the name *"Multistar 24FISH"*. This further inspired a successful Knowledge Transfer Partnership (KTP) supported by another fluorescent probe company CytoCell (www.cytocell.com) through which a series of non-human probes are currently being developed. The first "mouse Octochrome" is now in production, with several more including probes for distinguishing pig and avian chromosomes to be rolled out in 2014. The Bridge Centre financially supported Karyomapping development, which was subsequently taken on by BlueGnome (www.cambridgebluegnome.com) where it is set to become a centrepiece of their product range.

3. Enterprise activity: A patent for Karyomapping was granted in 2008 [*see 5.4*] and subsequently the research underpinning it was published by Handyside and Griffin [*see 3.4 above*]. The patent was sold to BlueGnome in 2011 [*see 5.5*]. Three successful Technology Strategy Board (TSB) including the KTP (above) grants (total value over £1.7 million) support the animal work including contributions from JSR Genetics (www.jsr.co.uk), The Bridge Centre, Paragon Vets (www.paragonvet.com/), Cogent (www.cogentuk.com), Cytocell, and Illumina (www.illumina.com/ BlueGnome's parent company) [*see Section 3- Major grants*]. This level of investment by the companies is indicative of the value the agricultural sector places on this technology.

4. Animal breeding industry, including potential environmental benefits: Animal PGD/S work is in its infancy, and the rationale for funding is that it has the potential to benefit the environment through reduction in greenhouse gas emissions. JSR genetics has re-configured its business model to focus on the sale of the genetic merit of the animal (this could include an embryo) rather than the animal itself as a result of our collaborative work [*see 5.6*]. Building on the Karyomapping technology, a computer-based tutorial called "KaryoLab" has been developed by Griffin and is now used in some 20 universities worldwide *("...we would be lost without it.."* Dr Terry Robinson University of Stellenboch, South Africa). A variant of KaryoLab (KaryolabPorc) has also been employed in pig breeding programmes. "....*KaryoLabPorc has had a greater than anticipated effect in that we have succeeded in eradicating chromosome translocations from our breeding herd completely.."* Dr Alan Mileham, Genus plc [*see 5.7*]. This herd comprises some 120,000 piglets per year.

5. Training & job creation: Handyside and Griffin continue to work closely with BlueGnome with Handyside being a part-time BlueGnome employee. Five Kent students have gained full employment with the Bridge Centre and major TSB and KTP grants awarded to Griffin [*see 3 above*] have employed three Kent PhD graduates as research associates. Furthermore, the Bridge Centre supported one Kent PhD student directly, while six others (and one undergraduate) gained work experience in their genetic clinical laboratories. Three part time Kent PhD students are currently Bridge Centre or BlueGnome employees. JSR Genetics, Pfizer, Genus plc (http://www.genusplc.com) and Digital Scientific [*see 5.8*], all supported BBSRC CASE studentships with Griffin as the academic supervisor.

6. Influence on public opinion and policy: The Human Fertilization and Embryology Authority (HFEA) is considering the impact of Karyomapping. Quote: "[*HFEA*] *highlighted Karyomapping.. as a high priority for discussion following horizon scanning*" [*see 5. 2*]. The Karyomapping project was shortlisted by the Times Higher Education (THE) as Research Project of the Year in 2010 [*see 5.9*]. The project has also received a significant level of coverage in both the national press e.g. The Telegraph [*see 5.10*] and broadcast media e.g. BBC1/ITV local and national news bulletins.

7. Education: The underpinning research has been incorporated into undergraduate programmes and has inspired the University of Kent to launch, in 2011, a taught MSc programme in

Reproductive Medicine. Graduating students (5 in 2012 and 11 in 2013) have benefitted from the research that underpins this case and 5 are currently employing it in their subsequent careers.

8. Public engagement: Griffin and Handyside engage extensively with the public: Karyomapping (and PGD/S generally) has been incorporated into more than 50 public lectures in a wide range of public forums including Café Scientifique, Rotary Clubs, medical societies, prospective undergraduates, science fairs, schools, patient groups and webinars including one by Griffin for the American Chemical Society (http://acswebinars.org/designerbabies).

5. Sources to corroborate the impact

5.1: A report on the first clinical application of karyomapping for preimplantation genetic diagnosis of Gaucher Disease combined with 24 chromosome screening, presented at the 10th International Congress on Preimplantation Genetic Diagnosis and published as an abstract in Reproductive Biomedicine Online - 20. DOI:10.1016/S1472-6483(10) 62301-2.

5.2: Letter dated 19th September 2013 from a member of the Human Fertilisation and Embryology Authority (HFEA), confirming that the HFEA are currently assessing the impact of the Karyomapping technology.

5.3: Product details of Multistar 24FISH and Cytocell animal probes www.kreatech.com/rest/products/repeat-freetm-poseidontm-fish-probes/preimplantation-genetic-screening/multistar-24-fish.html and www.cytocell.co.uk/products/multiprobe/OctoChrome-Paints/

5.4: Patent "*Chromosomal Analysis by Molecular Karyotyping*"; describing the Karyomapping method of molecular karyotyping: US application publication no. 2008/0318235 European patent EP1951897B1; India patent application no. 2390/KOLNP/2008; Japanese patent publication no. JP5178525B2.

5.5: Press release dated 15th October 2010 confirming the acquisition by BlueGnome of the intellectual property for Karyomapping. See www.cambridgebluegnome.com/news/prof-alan-handyside-brings-his-karyomapping-to-bluegnome

5.6: "Success for JSR at University of Kent Innovation Awards"; a press release issued in Oct 2012 by JSR Genetics, one of the largest family-owned farming companies in the UK. See: www.jsrgenetics.com/news/success-for-jsr-at-university-of-kent-innovation-awards. The Director of Science and Technology at JSR Genetics can also confirm that his company has reconfigured its business model as a result of their collaborative work with Griffin.

5.7: Letters of support for the educational value of KaryoLab including one from the Head of Genomics, Genus plc, confirming that that his company's use of KaryoLabPorc has had a significant impact on the management of pig breeding programmes and increased the profitability of the company.

5.8: Article on the Digital Scientific UK web site highlighting the company's collaboration with the Griffin laboratory at Kent and the Griffin-supervised PhD students sponsored by the company. See http://www.dsuk.biz/DSUK/Collaborations.html.

5.9: Press release entitled "University of Kent shortlisted for two Times Higher Education Awards" confirming that the Karyomapping project was Research Project of the Year nominated for Times Higher Research Project of the Year (2010).
www.kent.ac.uk/news/stories/timeshigherawards/2010

5.10: Example of press coverage of the potential of karyomapping technology: The Telegraph, Oct 2008 (www.telegraph.co.uk/health/3250003/Groundbreaking-test-will-screen-embryos-for-genetic-disorders.html).