Institution: University of Kent

Unit of Assessment: 5: Biological Sciences

Title of case study: Novel Chromosome Screening Tools in Assisted Reproduction Technology (ART) Enhance Human Fertility Treatment and Animal Breeding Regimes for Food Production

Period when the underpinning research was undertaken: 2006-2020

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:
Darren Griffin	Professor of Genetics	2004-present
Alan Handyside	Professor of Reproductive Genetics	2013-2014 (0.2 FTE) 2014-present (honorary)
Rebecca O'Connor	Research and Business Development Fellow	2016-2020

Period when the claimed impact occurred: 2014-2020

Is this case study continued from a case study submitted in 2014? No

1. Summary of the impact

University of Kent research on the genetics of infertility and disease enabled the invention and adoption of two novel chromosome screening tools: Karyomapping and Enhanced Translocation Screening. Karyomapping is a universal method to diagnose any genetic disease in human IVF embryos. It has been successfully utilised worldwide, treating >15,000 couples since 2014. The ease with which Karyomapping can be applied has a positive impact on patient care. It reduces the time from first admission to treatment commencement, thereby relieving stress and anxiety. Karyomapping was, in **2017,** adapted for use in cattle (~2000 embryos screened so far) to improve pregnancy rates following IVF. In parallel, an Enhanced Translocation Screening method was developed and adopted to identify sub-fertile stud males in pig and cattle breeding, to date screening >2,000 elite animals. It removes a major cause of reduced litter size by eliminating affected animals from breeding programmes, thereby also lowering the carbon footprint. Impact from Karyomapping and Enhanced Translocation Screening was achieved by improving assisted reproduction technology (ART), advancing business performance in fertility clinics and agriculture, improving food production, and informing professional practice.

2. Underpinning research

Assisted reproduction technology (ART), chiefly comprising *in vitro* fertilisation (IVF), embryo transfer, and artificial insemination, is at the core of both human infertility treatment and modern agriculture. Underpinning research by Griffin and Handyside has, for over 30 years, aimed to identify IVF embryos (in humans and other animals) that are free of genetic disorders.

In a medical context, transfer of genetically abnormal embryos can cause either unsuccessful treatment cycles, pregnancy loss, or the birth of children with severe diseases. Griffin and Handyside have been at the vanguard of technologies designed to avoid this (so-called preimplantation genetic testing [PGT]) from the outset. Traditionally, PGT employed bespoke tests either for abnormalities in the number of chromosomes in a cell (aneuploidy) (this is called PGT-A); or for monogenic (single gene) disease (this is called PGT-M); but not for both together. Moreover, PGT-M previously typically required at least six months of patient 'work-up' to adapt the test to the specific couple's needs. Griffin and Handyside first published together on PGT in 1991

and, around **2006**, for the first time, developed strategies based on the interpretation of information provided by single nucleotide polymorphisms (DNA sequence variants that can be used as markers to track inheritance of disease genes). This collaborative work combined first-hand experience of addressing clinical needs with research tools and ideas available in the Kent Griffin lab; these were essential for converting basic research to working solutions. The work published in **2010** identified a novel strategy (Karyomapping), combining information on single nucleotide polymorphisms from parents and siblings of an embryo in such a way that it enabled detection of any monogenic disorder (PGT-M) and any aneuploidy (PGT-A) in a single and universal assay. This was a substantial advance on state-of-the-art techniques of the time and requires no prior patient 'work-up'.

Following publication of the initial article **[R1]** (Handyside first author, Griffin senior (last) author), Handyside was appointed to a 0.2 FTE position at Kent. In **2014**, the Kent team reported the first application of Karyomapping to support IVF cycles in a clinical setting **[R2]**. This produced healthy, non-aneuploid embryos, despite a pre-existing parental risk for a severe monogenic disorder **[R2]**. In subsequent work, the technology was validated in a larger group of patients **[R3]**, then rapidly taken up by IVF clinics and the labs that service them.

In cattle, IVF and PGT (using single nucleotide polymorphism information) are usually performed in high volumes from genetically elite parents. In this agricultural context, transfer of embryos detected to have a higher genetic merit (e.g. better meat quality, higher milk yield, greater disease resistance) improves the financial return achieved by the breeding programme. Shortened generation times and improved food conversion ratios (that lead to less waste) also have financial and environmental benefits. Underlying research in the Griffin lab, initiated in **2015**, sought to apply Karyomapping for PGT-A, for the first time, to improve cattle IVF success rates. Application of this technology published in **2019** led to the first live born calves **[R4]** and, to date, a highly significant (6%) increase in pregnancy rates. Karyomapping is now also being applied, experimentally, in a pig IVF lab set up by Griffin in **2012.**

In parallel to the development of Karyomapping, research in the Griffin lab sought to improve approaches to chromosomal diagnostics of parent animals. Chromosomal imbalance (numerical and structural anomalies) in the sperm of boars and bulls used for artificial insemination, has been an ongoing concern of the pig and cattle industry for over 40 years. Sires with balanced chromosome rearrangements (i.e. those that involve the interchange of chromosomal segments from two different chromosomes without loss of genetic material) are sub-fertile and typically produce chromosomally imbalanced sperm in large proportions. This leads to reduced pregnancy rates, pregnancy loss, and stillborn offspring. Traditional chromosome translocation screening (karyotyping) from the blood of elite sires has typically prevented the sperm of affected males entering ART breeding programmes. Griffin and O'Connor's research (**2014-present**) sought to address the problem that traditional karyotyping approaches fail to identify certain abnormalities (so-called cryptic translocations). Outcomes of the underpinning results led to the development of a unique Enhanced Translocation Screening approach. This uses multiple fluorescent probes to achieve chromosome-by-chromosome detection of sub-telomeric sequences as a means of highlighting the ends of each chromosome. It thereby facilitates the identification of rearrangements between chromosomes (translocations) **[R5, R6]**. Rapid adoption by leading European pig breeders (e.g. JSR, PIC, Topigs Norsvin) led to a programme of routine screening (to date >2,000 boars) and subsequent development of an analogous cattle device **[R7]**. Research demonstrated that this approach requires minimal analysis training (unlike traditional karyotyping, which requires specialist knowledge), and easily detects cryptic translocations, enabling up to twice as many abnormal boars **[R6]** and six times as many affected bulls **[R7]** to be identified.

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

The Griffin lab is comprised of Kent staff and PhD students (under his direct supervision), here indicated in **bold**.

[R1] Handyside, A. H., Thornhill, A. R., **Harton, G. L.,** Mariani, B., Shaw, M. A., Affara, N., and **Griffin, D. K**. (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. doi: 10.1136/jmg.2009.069971

[R2] Natesan, S., Handyside, A. H., Thornhill, A. R., **Ottolini, C. S.**, Sage, K., Summers, M. C., Gordon, A., Michaelis, Konstantidis M., Wells, D., and **Griffin, D. K**. (2014). 'Live birth after PGD with confirmation by a comprehensive approach (karyomapping) for simultaneous detection of monogenic and chromosomal disorders'. *Reproductive Biomedicine Online* 29: 600-605. doi: 10.1016/j.rbmo.2014.07.007

[R3] Natesan, S. A., Bladon, A. J., Coskun, S., Qubbaj, W., Prates, R., Munne, S., Coonen, E., Dreesen, J. C., Stevens, S. J., Paulussen, A. D., Stock-Myer, S. E., Wilton, L. J., Jaroudi, S., Wells, D., Brown, A.P., and Handyside, A. H. (2014). 'Genome-wide karyomapping accurately identifies the inheritance of single-gene defects in human preimplantation embryos in vitro'. *Genetics in Medicine* 16: 838-845. doi: 10.1038/gim.2014.45

[R4] Turner, K. J., Silvestri, G., Black, D., Dobson, G., Smith, C., Handyside, A. H., Sinclair, K. D., and **Griffin, D. K**. (2019). 'Karyomapping for simultaneous genomic evaluation and aneuploidy screening of preimplantation bovine embryos: The first live-born calves'. *Theriogenology* 125: 249-258. doi: 10.1016/j.theriogenology.2018.11.014

[R5] O'Connor, R. E., Fonseka, G., Frodsham, R., Archibald, A. L., Lawrie, M., Walling, G. A., and **Griffin, D. K**. (2017). 'Isolation of subtelomeric sequences of porcine chromosomes for translocation screening reveals errors in the pig genome assembly'. *Animal Genetics* 48: 395-403. doi: 10.1111/age.12548

[R6] O'Connor, R. E., Kiazim, L., Rathje, C. C., Jennings, R. L., and **Griffin, D. K**. (2021). 'Rapid Multi-Hybridisation FISH Screening for Balanced Porcine Reciprocal Translocations Suggests a Much Higher Abnormality Rate Than Previously Appreciated'. *Cells* 10,250. doi: 10.3390/cells10020250

[R7] Jennings, R. L., Griffin, D. K., and **O'Connor, R.E.** (2020). 'A New Approach for Accurate Detection of Chromosome Rearrangements That Affect Fertility in Cattle'. *Animals* (Basel) 10: 114. doi: 10.3390/ani10010114

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

Context

Research by the Kent team has provided solutions for preventing transmission of chromosomal and single-gene disorders in the context of ART (assisted reproduction technology). ART is used extensively in medicine for fertility treatment (1 in 6 couples are infertile) to prevent genetic disease (1 in 50 couples are at risk of transmitting a genetic disorder). ART is also used in modern meat production (>90% of the global population eat meat). In humans, >2,500,000 IVF treatment cycles worldwide result in 500,000 successful deliveries annually. In animal husbandry, transfer of IVF-derived cattle embryos generates large numbers of offspring from parents with elite breeding potential. In 2018, >700,000 IVF cattle embryos were produced and transferred. In pigs, IVF technology is not yet used in the breeding industry, but is being explored experimentally by the Kent team. In both pigs and cattle (as well as other farm animals), artificial insemination is the leading approach to disseminate superior genetics, with typically >100 million procedures (each for pigs and cattle) performed per annum worldwide. Applications resulting from Kent research (Karyomapping and Enhanced Translocation Screening) have led to significant impact in improving these critical areas. Impact has been in the areas of improved ART outcomes, advanced business performance in fertility clinics, agriculture, improved food production, educational and professional practice.

Commercial Impact on IVF service providers in clinical and agricultural settings

The Karyomapping patent was acquired by Illumina in 2013. In **2019**, Vitrolife acquired the rights to market and distribute Karyomapping services as one of their portfolio products, and the company's Senior Vice President commends the 'positive impact [Karyomapping] has both on IVF improvement in patient care and on our business' **[a, b]**. Vitrolife market Karyomapping services to diagnostic laboratories such as CooperGenomics **[c, d]** and Igenomix **[e]**. CooperGenomics processed over 10,000 Karyomapping clinical cases between **2015** and **2020**, and cites the number of cases performed worldwide at around 15,000 at time of writing **[c]**. Their Director of Clinical Operations describes Karyomapping as 'one of the most innovative tests in reproductive medicine' that has been incorporated into their marketing strategy, and 'has contributed to our profitability through the sales, enhanced business performance, and innovative activity it generates for our business' **[c]**. He further states that 'Karyomapping has allowed us to retain around 20 full time equivalent staff in gainful employment', and he expects this will 'grow in coming years; given the interest I see in [Karyomapping] at scientific meetings' **[c]**.

Igenomix, by their own estimation the 'leading lab for Karyomapping' in the UK, have tested 2000 embryos since gaining ISO accreditation in **2018 [e, f]**. Their UK Country Manager and Senior Scientific advisor, Alan Thornhill, describes Karyomapping as 'one of our flagship products', the adoption of which has been central to 'increasing the profitability and profile of the company' **[e]**.

Improving IVF Treatment Outcomes and Patient Welfare through Karyomapping

The primary aims of Karyomapping in medical applications are to maximise the chance for IVF patients to give birth to genetically healthy children and to improve IVF outcomes significantly **[d]**. Igenomix's Alan Thornhill attests to the 'impact that Karyomapping is having in terms of advancing the practice, outcomes and understanding in the field of reproductive technologies' **[e]**. Moreover, the benefits of Karyomapping extend beyond improving treatment outcomes.

The Global Vice-President and Director of Clinical Operations at CooperGenomics states that Karyomapping has 'revolutionised how comprehensively PGT-M can be offered to patients', meaning that the beneficial effect of PGT-M now helps more patients than with previous approaches **[c]**. The fact that, unlike previous approaches, Karyomapping can be applied 'without the need for long-term patient work-up' **[c]**, and that 'waiting time is minimal compared to previous approaches and now averages 4 weeks' **[e]**, means that patient stress is reduced and loss of time avoided. The development of Karyomapping has also contributed to advocacy for PGT, and has changed professional practice. In his role as former member of the UK's Human Fertilisation and Embryology Authority, Igenomix's Alan Thornhill describes Karyomapping as 're-shaping and redefining the provision of PGT-M – an internationally acclaimed regulatory success story' **[e]**. Evidence that Karyomapping is regularly discussed by the Human Fertilisation and Embryology Authority is provided in the document 'Embryo testing: Testing for more than one condition at a time', and in the minutes of the Scientific and Clinical Advances Advisory Committee (SCAAC) **[g]**.

Karyomapping and Enhanced Translocation Screening Advance Animal Breeding Practice

In a veterinary context, Karyomapping has been applied experimentally to 2,000 cattle embryos in collaboration with Paragon, a mixed species veterinary practice with over 25 veterinarians **[h]**. The company's Managing Director, David Black, highlights the value of this collaborative research to the company in stating that their 'submission to Companies House in April 2020 filed total company equity of £1,279,443, supported, in no small part, by high impact research activities', including activities such as those resulting from their eight-year collaboration with Prof. Griffin **[h]**. The collaborative research was so successful that it led to the establishment of a spin-out company, Activf-ET, which 'was created specifically to exploit and fully realise the impact of the findings [of the] TSB/Innovate projects undertaken' with its University partners **[h]**. Black further highlights how the research has underpinned company performance by enabling breeders to 'estimate the "Breeding Value" (through genomic analysis of the animal from which the embryo will arise)', and to 'assess whether the embryo is likely to implant, and proceed to a healthy pregnancy, or not' **[h]**. Black adds that 'such information is vital to the company as we can eliminate from our system those embryos unlikely to develop' **[h]**.

Following on from the development of Enhanced Translocation Screening through the Kent team's research **[R5-R7]**, and emerging evidence of the strong interest of this approach to the pig-breeding industry, the Kent team established an in-house enterprise activity for the screening of elite breeding boars under the name CytoScreen Solutions **[i, j]**. In **July 2020**, the set-up had serviced 15 companies from 10 European countries. Blood samples from boars are received and subjected to Enhanced Translocation Screening by the Kent team, with the aim of identifying boars where chromosome translocations indicate sub-fertility. Evidence on the impact of CytoScreen Solutions' services is provided by two of these companies. British JSR Genetics, 'the largest UK based supplier of pig genetics', work in a consortium with Dutch company Topigs Norsvin to provide 'top-quality breeding stock and semen', thereby 'providing pigs that perform profitably for the producer' **[i]**. PIC USA Inc., 'one of the world's leading providers of genetic material to the global pig industry', is the porcine genetics branch of US company Genus plc **[j]**. PIC contributes over £250 million to the parent company's revenue by providing 150 million market pig equivalents to more than 2500 customers.

PIC have fully replaced their karyotyping-based procedures preceding the artificial insemination of sows with CytoScreen Solutions' Enhanced Translocation Screening **[j]**. PIC provide a detailed estimate of the financial impact of this, stating that 'failing to identify [sub-fertile boars] could mean up to £50,000 in production losses per individual male' **[j]**. They also estimate that Griffin's 'novel approach detects 1.5-2x more' than the published incidence of sub-fertile boars, thus helping PIC to detect 'over 70 animals to date' **[j]**.

JSR Genetics state that 'all JSR damlines and Topigs Norsvin nucleus boars as well as boars from our partners HKScan are screened to ensure that they are not carrying chromosomal translocations' by Cytoscreen Solutions **[i]**. They also confirm that hypoprolific boars carry a negative 'financial impact of ~£45,000 to the business', and that with Translocation Screening 'we have been able to detect translocations that we would fail to identify using the older conventional approach' **[i].** The novel approach thus avoids financial losses worth millions of pounds, as well as avoiding 'significant reputational risk to our commercial operations', 'contract defaults', and likely reducing the additional carbon footprint produced by raising boars with reduced fertility **[i]**. A cattle version of Enhanced Translocation Screening is now in production through CytoScreen Solutions, with over 100 bulls screened to date.

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

[a] Letter of support from Vitrolife Senior Vice-President and Head of Genomic Business Unit.

[b] Promotional material from VitroLife, providing evidence that Karyomapping is a product offered commercially.

[c] Letter of support from CooperGenomics, signed by Global Vice-President and Director of Clinical Operations.

[d] Promotional material from CooperGenomics, providing evidence that Karyomapping is a product offered commercially.

[e] Letter of support from Igenomix, signed by UK Country Manager and Senior Scientific Advisor.

[f] Evidence of ISO accreditation awarded to Igenomix for the use of Karyomapping.

[g] Human Fertilisation and Embryology Authority document: 'Embryo testing: Testing for more than one condition at a time', including minutes of the Scientific and Clinical Advances Advisory Committee (SCAAC) in which Karyomapping was discussed (pp. 2, 3, 4, 7, 31, 40).

[h] Letter of support from Paragon, signed by the Managing Director.

[i] Letter of support from Topigs Norsvin, JSR Genetics Meat Group Leader and Research Director.

[j] Letter of support from PIC USA, Inc., signed by the Global Director of Product Development.