
Article:
Huntriss, J, Balen, AH, Sinclair, KD et al. (3 more authors) (2018) Epigenetics and Reproductive Medicine. BJOG: An International Journal of Obstetrics and Gynaecology, 125 (13). E43-E54. ISSN 1470-0328

https://doi.org/10.1111/1471-0528.15240

Scientific Impact Paper No. XX
Final Draft – October 2017

Epigenetics and Reproductive Medicine

1. Introduction

In 1942, Conrad H Waddington introduced the term 'epigenetics', to describe a biological process that takes place between the genotype and phenotype.[1] Epigenetics was subsequently defined as 'the study of mitotically and meiotically heritable changes in gene function that cannot be explained by changes in DNA sequences'.[2] It is a gene-marking and gene-regulatory system that is essential for normal mammalian development. Examples of epigenetic marks include DNA methylation[3] and covalent modifications that are positioned on the histone proteins, the 'histone code', that act to regulate chromatin function.[4] Of importance to the field of reproductive medicine, epigenetic marks are extensively reprogrammed during gametogenesis and preimplantation embryonic development. These epigenetic modifications, in addition to RNA-based epigenetic mechanisms,[5] are important in regulating gene expression.[6] The appropriate regulation of epigenetic information is critical to normal development, since the disruption of epigenetic mechanisms can cause disease.[7–11]

2. Epigenetics in reproduction, development and reproductive medicine

The natural periods during which developmental epigenetic reprogramming in gametes and preimplantation development occur coincide closely with the time during human assisted reproduction that the gametes and embryos are being handled in an in vitro environment. The best understood epigenetic reprogramming cycle is that of DNA methylation. The lifecycle of this epigenetic mark includes several key stages including: the erasure of epigenetic marks from primordial germ cells; the establishment of a new set of marks during gametogenesis; genome-wide erasure of methylation during the preimplantation stages; and *de novo* establishment of marks during development and differentiation from around the blastocyst stage (that is day 5 of embryo development) onwards.[12,13] Newly-identified processes that act to erase DNA methylation from primordial germ cells and during preimplantation development have been detected.[14,15] Currently, it is not possible to assess the epigenetic status of the human preimplantation embryo during routine assisted reproductive technology (ART). It is not at this time, therefore, possible to deduce:

- whether epigenetic defects exist unequivocally in ART-derived embryos, and
- what effects any putative ART-induced epigenetic changes will have upon the growth, development and health of the conceptus.

This review will summarise current viewpoints on our understanding of epigenetics and the relevance of these findings to reproductive medicine.

3. Genomic imprinting

Genomic imprinting is a system of gene expression used in mammals, plants and insects that is controlled by epigenetic information[16] and is limited to a restricted number of genes.[17] It can be defined as the exclusive or predominant expression of one allele of a gene (from either the maternal or paternal allele, depending on the gene in question). For example, the insulin-like growth factor II gene is an imprinted gene expressed from the paternal allele, while the *H19* gene is an imprinted gene expressed from the maternal allele. This monoallelic expression is regulated

by allele-specific epigenetic marks, such as DNA methylation, which are established in the germline and, importantly, are actively maintained during preimplantation development to allow continued marking and appropriate monoallelic expression of the correct parental allele of the imprinted gene. Imprinted genes are particularly important in the regulation of energy balance between the mother and the developing fetus via the placenta,[18,19] and current hypotheses suggest that genomic imprinting may allow the exertion of parental epigenetic influences on the growth and development of the conceptus.[20,21] Correct imprinted gene transcript dosage is critical for early development.[22] Over 200 imprinted genes have been described to date in humans, with many imprinted genes locating to clusters on the chromosomes.[23,24] In humans there are a number of congenital disorders, termed imprinting disorders (IDs), caused by the disruption of imprinted genes, including Beckwith-Wiedemann syndrome (BWS), Silver-Russell syndrome (SRS), and Angelman syndrome (AS).[25] Of these, BWS and SRS appear to be associated with assisted reproduction.[26–28]

4. Disorders of genomic imprinting and human assisted reproduction

A systematic review and meta-analysis of the literature has revealed that the risk of IDs is higher in children conceived through assisted reproduction (in vitro fertilisation [IVF] or intracytoplasmic sperm injection [ICSI]) than in those conceived naturally.[29] Summarising data from eight epidemiologic studies of BWS and ART, Vermeiden and Bernardus[30] reported a significant positive association between IVF/ICSI treatment and BWS, and described a relative risk of 5.2 (95% CI 1.6–7.4), indicating that one BWS child will be born for every 2700 IVF/ICSI births when using a population prevalence in the general population of 1:13 700. The same report concluded that there probably is a significant positive association between the incidences of SRS and IVF/ICSI treatment, but noted that the number of published cases is small (13 SRS children born after ART). It is important, therefore, to note that while cases of IDs are rare, it is necessary to understand how ART causes epigenetic disruption in case these outcomes are sentinel indicators of more widespread epigenetic disruption, which may include non-imprinted loci.

5. Epigenetic changes attributed to assisted reproduction procedures

In addition to experimental data from other mammals, there is evidence from human studies that a number of assisted reproduction procedures, including superovulation, micromanipulation, in vitro maturation of oocytes and embryo culture, can cause epigenetic disruption.[31–33] Unfortunately, assisted reproduction procedures are performed at a time when dynamic, essential epigenetic reprogramming events are occurring in the gametes and embryos, yet the extent of these epigenetic changes and the relevance to human health and disease in assisted reproduction cohorts is only just beginning to be understood. It is important, therefore, that the use of assisted reproduction should be closely monitored.[34] Two assisted reproduction procedures will be discussed in detail here as examples of how these may lead to epigenetic disturbance.

5.1 In vitro culture of embryos

A large number of publications have described the effects of in vitro culture (IVC) on gene expression in preimplantation embryos from several mammalian species.[31,35,36] The expression and/or methylation of a number of imprinted genes are disrupted by IVC in some, but not all, types of culture media.[37–41] Arguably the most comprehensive assessment to date was reported by Schwarzer et al.,[42] who demonstrated that culture media can induce a wide range of cellular, developmental and metabolic changes in mouse preimplantation embryos, including effects on metabolic pathways, a conclusion reinforced by Gad et al.[43] Very few studies have investigated the effects of culture media in human preimplantation embryos. Kleijkers et al.[44] reported that

genes from several pathways were differentially expressed in the two media tested (G5 medium and human tubal fluid medium). In a more recent study by Mantikou et al.[45] 174 genes were differentially expressed in human embryos cultured using these same two media. Given the current interest in developing embryo culture media that contain growth factors, it is also worth noting that Kimber et al.[46] showed that single growth factors added to human embryos in culture caused unexpected changes in gene expression profiles. In contrast, a histological study in mice reported that the appearance of the placentas or fetuses derived from embryos cultured in different media did not differ, however, this study did not involve molecular analysis.[47] A further example of the detrimental effects of IVC is illustrated by large offspring syndrome (LOS), which may be observed after IVC in ruminants and results in the fetus growing large in the uterus, bringing risks to the mother as well as the offspring.[48] In a comprehensive genetic analysis using RNA sequencing, LOS was revealed to involve a multi-locus loss of imprinting syndrome.[49] These studies highlight that in some circumstances, IVC has the potential for inflicting genome-wide changes in gene expression/methylation that can have developmental consequences.

5.2 Evidence for the influence of in vitro culture on human birthweight

Birthweight is an important metric as it is a useful, routinely collected surrogate for fetal growth and, along with early postnatal growth, a strong predictor of the long-term risk of cardiometabolic disease.[50,51] In a comparative study of two commercially available media used for IVC of fresh embryos, Dumoulin et al.[52] reported a significant difference in birthweight (3453 ± 53 g [sample error of the mean] versus 3208 ± 61 g, $P = 0.003$) and in birthweight adjusted for gestational age and gender. Similar findings were reported in a subsequent study from the same group[53] performed in a larger cohort. Furthermore, differences in postnatal weight were observed during the first 2 years of life.[54] In another study, no significant differences in mean birthweight or mean birth length were reported comparing three other types of embryo culture media.[55] Further studies[56–58] using a range of media also failed to reveal significant differences in birthweight. Other culture conditions that might affect birthweight are the age of the media,[59] the length of the culture period (relevant to the extended culture periods used in blastocyst culture versus cleavage-stage transfer),[60] and the protein source used in the media.[61] These studies were summarised by Zandstra et al.,[50] who concluded that of 11 media comparisons published, six showed differences in birthweight while five did not. The list of culture conditions presented is not necessarily complete and it is possible that other factors may be identified in the future. A working party of the European Society for Human Reproduction and Embryology has called for national assisted reproductive technology (ART) registries to track culture media used, to allow the long term assessment of health risk, and encourage full disclosure of media composition by commercial manufacturers.[62] From this report, a number of recommendations were made including:

1. A requirement for openness from manufacturers regarding any media formulation changes and the scientific rationale for any changes.
2. The use of quality management systems by ART clinics to ensure that culture medium is stored and used correctly.
3. Clinic follow-up of the health of the offspring as a quality control measure.
4. A record of the type of culture medium used be recorded in the national register.

The influence of media on pregnancy and perinatal outcome after IVF has also been considered in a randomised control trial, published in 2016.[63] This study compared outcomes after embryo culture in either G5 or human tubal fluid media and reported that birthweight was significantly lower in the G5 group while the clinical pregnancy rate was significantly higher. Although the

findings of this study were considered controversial by some sectors of industry, they were recently corroborated by an independent statistical analysis. [64]

5.3 Controlled ovarian hyperstimulation/superovulation

Data from animal and human studies indicate that the process of ovarian stimulation may induce epigenetic errors in the oocyte, embryo and placenta. Controlled ovarian hyperstimulation (COH)/superovulation overrides the progressive, oocyte growth-dependent process of epigenetic maturation and imprint establishment,[65,66] or may lead to the recruitment of poor quality oocytes that would not normally be selected to ovulate.[67,68] COH in humans is associated with epigenetic changes at a small number of tested loci[69,70] and was reported as the only common factor in the medical records of women who gave birth tochildren with BWS after ART.[71] Mouse studies have identified transgenerational effects of superovulation,[72] with epigenetic changes persisting in the sperm of the second generation offspring of superovulated mothers. Superovulation has also been reported to cause perturbed genomic imprinting of maternally- and paternally-expressed genes in the embryo and placenta,[68,73] and is therefore likely to disrupt key oocyte/early embryo-specific factors important for imprint maintenance during preimplantation development.[74–76]

6. The evidence for epigenetic changes in human assisted reproductive technology embryos

Epigenetic errors have been reported to be inherent in arrested human embryos.[77] Several studies have indicated that imprinted genes such as *SNRPN, H19, PEG1/MEST, KCNQ1OT1* and imprinted gene regulatory regions in some human preimplantation embryos may be susceptible to abnormal DNA methylation patterns or gene expression patterns.[78–81] Such studies include analysis of KvDMR1 [1], the DMR that is aberrantly methylated in ART-related BWS in humans, and is hypomethylated in LOS following assisted reproduction in bovine embryos.[82–86] However, the merits of attempting to measure 'epigenetic health' with methylation data obtained from such a restricted number of loci is currently limited, since there is insufficient knowledge of developmental epigenetic processes in humans to demonstrate conclusively whether any particular epigenetic defect detected in the preimplantation embryo will cause disease in the infant at birth or might be manifest later in development.

7. Infertility and epigenetics

In addition to effects induced by ART, it is important to consider cases of infertility in which gametogenesis itself is susceptible to epigenetic defects. Perturbed epigenetic signatures in sperm are observed in cases of male infertility,[87,88] and epigenetic screening of sperm may be of potential use clinically.[89–91] There may be equivalent epigenetic defects in the female germline associated with female infertility. Kobayashi et al.[92] indicated that in some cases epigenetic errors may be inherited from the sperm, but other studies suggest that epigenetic defects are due to the procedure itself rather than defects in the gametes.[79,80,93] It remains possible that pre-existing gametic epigenetic defects could be exacerbated by suboptimal conditions in assisted reproduction. Other features of couples presenting for ART must also be considered, for example, advanced age, diet, body composition, environmental exposures and genetic/epigenetic variation which have all been shown to affect epigenetic programming in the mammalian germline.[87,94–98]

8. The evidence for epigenetic changes in human assisted reproduction cohorts

[1] KvDMR1: An intronic CpG island within the *KCNQ1* gene and *KCNQ1OT1* gene.

The epigenetic profiles of ART cohorts appear to differ from those naturally conceived, as summarised by Batcheller et al.[99] However, studies have been limited by the type of assay used, its coverage of the genome and the type of cell used for analysis. In more recent work, quantitative assessment of methylation indicated that use of ICSI was associated with a higher level of *SNRPN* methylation.[100] In another study, Melamed et al.[101] used a methylation array, which allows wider sampling of the genome, and revealed that hypomethylation was observed in the assisted reproduction group. It was concluded that ART may be associated with significantly higher variation in DNA methylation compared with natural conception, in agreement with other studies.[27]

9. Adult cardiovascular and metabolic diseases: a partial legacy of assisted reproduction?

Several studies have indicated that ARTs are associated with fetal growth restriction (FGR), prematurity, low birthweight for gestational age, and slightly increased risk of cardiovascular malformations and other defects.[102,103] A long term follow-up study suggested a potential increase in the incidence of elevated blood pressure and fasting glucose, and increased total body fat in IVF offspring (reviewed in Hart and Norman[104]). Systemic and pulmonary vascular dysfunction[105] and right ventricular dysfunction[106] have been observed in children and adolescents conceived through ART. Assisted reproduction may also lead to cardiac and vascular remodelling that persists in human fetal and postnatal development.[107] Cardiovascular and metabolic effects are also seen in mouse studies where there is evidence for an epigenetic origin for these problems.[108] Thus, in mice conceived by IVF epigenetic changes were observed at imprinted genes, alongside methylation and expression of the endothelial nitric oxide synthase gene and arterial function in the aorta. Other studies support this growing body of evidence that there may be increased risks for metabolic and cardiovascular diseases following ART.[109–114]

It is possible that these outcomes are a result of the alteration/adaptation of metabolic pathways in mammalian embryos exposed to suboptimal culture media and/or environments.[42–44] Indeed, many enzymes involved in epigenetic gene regulation in eukaryotic cells make use of co-substrates and co-factors generated by cellular metabolism, thereby providing a direct link between culture environment and gene regulation.[115] Examples include cellular fluctuations in acetyl coenzyme A and histone acetylation, nicotinamide adenine dinucleotide and sirtuin deacetylase activity, and S-adenosylmethionine and histone/DNA methylation. Of these metabolic intermediaries, disturbances to S-adenosylmethionine-mediated epigenetic regulation during embryonic development has been the most comprehensively studied, influenced as it is by inputs into 1-carbon metabolic pathways.[116] These inputs include a diverse range of B vitamins (e.g. B12, folate [B9] and B6) and elements such as sulphur, zinc and cobalt. These in turn are influenced by lifestyle factors including obesity, cigarette smoking, alcohol and caffeine consumption,[117] which can lead to epigenetic dysregulation of gene expression in fetal tissues during early pregnancy.[118] The accumulating evidence indicates that a more holistic approach is required when offering guidance to couples undergoing fertility treatment that extends to dietary advice and lifestyle choices although clearly, further research is required. Strategies that avoid excessive use of ART should be considered.

10. Long-term effects of assisted reproduction on placental function

Assisted reproduction pregnancies have been associated with larger placentas and higher placental weight/birthweight ratios[119] in addition to modified imprinted gene expression and/or methylation in the placenta[120] and cord blood.[121] Such findings are likely to be important since imprinted genes are highly expressed and play a pivotal role in placental function.[122] In mouse experiments, ART can lead to multiple detrimental effects in the placenta[39,123–126] which

collectively provide molecular evidence that assisted reproduction can adversely affect placental function, with the potential to influence long term health.[127]

11. Opinion

- At least two disorders of genomic imprinting, BWS and SRS appear to be associated with ARTs, however the occurrence of these disorders is very rare.
- Evidence from a large number of animal studies reveals that ARTs including embryo culture, superovulation, in vitro maturation of oocytes, micromanipulation and embryo transfer have the potential to produce epigenetic changes that can cause dysfunction in the conceptus or placenta.
- A small number of human studies show that although ARTs, such as superovulation and cell culture, can induce epigenetic changes in the gametes and/or preimplantation embryo, the developmental effects of these changes and their involvement in disease process are currently unknown.
- Whether epigenetic disturbance is caused by ARTs or an epigenetic error in the gametes is unclear, but it is possible that in some cases assisted reproduction exacerbates pre-existing defects in the gametes.
- Further studies are required on whether the use of different culture media can affect birthweight in humans and on the possible effects of extended embryo culture. In agreement with the opinion of others,[63] and in view of the findings from the first randomised controlled trial on the effects of culture media on pregnancy and perinatal outcome,[64] greater transparency is essential with respect to the composition of embryo culture media.
- There is evidence for epigenetic differences and gene expression changes in ART cohorts when compared with those naturally conceived, although genome-wide studies are required to confirm this.
- Emerging data indicate that long-term consequences of ARTs may include cardiovascular and metabolic disorders, which may be due to compromised placental function.
- More research is required to ascertain the impact of ARTs and infertility on epigenetic programming on the human conceptus and any short-term and/or long-term developmental consequences that follow.

References

1. Waddington CH. The epigenotype. *Endeavor* 1942;1:18–20. Reprinted in *Int J Epidemiol* 2012;41:10–13.
2. Russo VEA, Martienssen RA, Riggs AD. Epigenetic mechanisms of gene regulation. Monograph 32. Plainview, New York, USA: Cold Spring Harbor Laboratory Press; 1996.
3. Klose RJ, Bird AP. Genomic DNA methylation: the mark and its mediators. *Trends Biochem Sci* 2006;31:89–97.
4. Bannister AJ, Kouzarides T. Regulation of chromatin by histone modifications. *Cell Res* 2011;21:381–95.
5. Holoch D, Moazed D. RNA-mediated epigenetic regulation of gene expression. *Nat Rev Genet* 2015;16:71–84.
6. Goll MG, Bestor TH. Eukaryotic cytosine methyltransferases. *Annu Rev Biochem* 2005;74:481–514.
7. Hirst M, Marra MA. Epigenetics and human disease. *Int J Biochem Cell Biol* 2009;41:136–46.
8. Wang J, Tang J, Lai M, Zhang H. 5-Hydroxymethylcytosine and disease. *Mutat Res Rev Mutat Res* 2014;762:167–75.
9. Kanwal R, Gupta K, Gupta S. Cancer epigenetics: an introduction. *Methods Mol Biol* 2015;1238:3–25.

10. Liyanage VR, Jarmasz JS, Murugeshan N, Del Bigio MR, Rastegar M, Davie JR. DNA modifications: function and applications in normal and disease states. *Biology (Basel)* 2014;3:670–723.
11. Weber W. Cancer epigenetics. *Prog Mol Biol Transl Sci* 2010;95:299–349.
12. Tomizawa S, Nowacka-Woszuk J, Kelsey G. DNA methylation establishment during oocyte growth: mechanisms and significance. *Int J Dev Biol* 2012;56:867–75.
13. Marcho C, Cui W, Mager J. Epigenetic dynamics during preimplantation development. *Reproduction* 2015;150:R109–20.
14. Yamaguchi S, Hong K, Liu R, Inoue A, Shen L, Zhang K, et al. Dynamics of 5-methylcytosine and 5-hydroxymethylcytosine during germ cell reprogramming. *Cell Res* 2013;23:329–39.
15. von Meyenn F, Reik W. Forget the parents: epigenetic reprogramming in human germ cells. *Cell* 2015;161:1248–51.
16. Macdonald WA. Epigenetic mechanisms of genomic imprinting: common themes in the regulation of imprinted regions in mammals, plants, and insects. *Genet Res Int* 2012;2012:585024.
17. Glaser RL, Ramsay JP, Morison IM. The imprinted gene and parent-of-origin effect database now includes parental origin of de novo mutations. *Nucleic Acids Res* 2006;34:D29–31.
18. Renfree MB, Suzuki S, Kaneko-Ishino T. The origin and evolution of genomic imprinting and viviparity in mammals. *Philos Trans R Soc Lond B Biol Sci* 2013;368:20120151.
19. Tunster SJ, Jensen AB, John RM. Imprinted genes in mouse placental development and the regulation of fetal energy stores. *Reproduction* 2013;145:R117–37.
20. Moore T, Haig D. Genomic imprinting in mammalian development: a parental tug-of-war. *Trends Genet* 1991;7:45–9.
21. Wolf JB, Hager R. A maternal-offspring coadaptation theory for the evolution of genomic imprinting. *PLoS Biol* 2006;4:e380.
22. Charalambous M, Ferron SR, da Rocha ST, Murray AJ, Rowland T, Ito M, et al. Imprinted gene dosage is critical for the transition to independent life. *Cell Metab* 2012;15:209–21.
23. Morison IM, Reeve AE. A catalogue of imprinted genes and parent-of-origin effects in humans and animals. *Hum Mol Genet* 1998;7:1599–609.
24. Skaar DA, Li Y, Bernal AJ, Hoyo C, Murphy SK, Jirtle RL. The human imprintome: regulatory mechanisms, methods of ascertainment, and roles in disease susceptibility. *ILAR J* 2012;53:341–58.
25. Eggermann T, Perez de Nanclares G, Maher ER, Temple IK, Tumer Z, Monk D, et al. Imprinting disorders: a group of congenital disorders with overlapping patterns of molecular changes affecting imprinted loci. *Clin Epigenetics* 2015;7:123.
26. Odom LN, Segars J. Imprinting disorders and assisted reproductive technology. *Curr Opin Endocrinol Diabetes Obes* 2010;17:517–22.
27. Hiura H, Okae H, Chiba H, Miyauchi N, Sato F, Sato A, et al. Imprinting methylation errors in ART. *Reprod Med Biol* 2014;13:193–202.
28. Hiura H, Okae H, Miyauchi N, Sato F, Sato A, Van De Pette M, et al. Characterization of DNA methylation errors in patients with imprinting disorders conceived by assisted reproduction technologies. *Hum Reprod* 2012;27:2541–8.
29. Lazaraviciute G, Kauser M, Bhattacharya S, Haggarty P, Bhattacharya S. A systematic review and meta-analysis of DNA methylation levels and imprinting disorders in children conceived by IVF/ICSI compared with children conceived spontaneously. *Hum Reprod Update* 2014;20:840–52.
30. Vermeiden JP, Bernardus RE. Are imprinting disorders more prevalent after human in vitro fertilization or intracytoplasmic sperm injection? *Fertil Steril* 2013;99:642–51.
31. Denomme MM, Mann MR. Genomic imprints as a model for the analysis of epigenetic stability during assisted reproductive technologies. *Reproduction* 2012;144:393–409.
32. Anckaert E, De Rycke M, Smitz J. Culture of oocytes and risk of imprinting defects. *Hum Reprod Update* 2013;19:52–66.

33. Fauque P. Ovulation induction and epigenetic anomalies. *Fertil Steril* 2013;99:616–23.
34. Kamphuis EI, Bhattacharya S, van der Veen F, Mol BW, Templeton A; Evidence Based IVF Group. Are we overusing IVF? *BMJ* 2014;348:g252.
35. Khosla S, Dean W, Reik W, Feil R. Culture of preimplantation embryos and its long-term effects on gene expression and phenotype. *Hum Reprod Update* 2001;7:419–27.
36. Huntriss J, Picton HM. Epigenetic consequences of assisted reproduction and infertility on the human preimplantation embryo. *Hum Fertil (Camb)* 2008;11:85–94.
37. Sasaki H, Ferguson-Smith AC, Shum AS, Barton SC, Surani MA. Temporal and spatial regulation of H19 imprinting in normal and uniparental mouse embryos. *Development* 1995;121:4195–202.
38. Doherty AS, Mann MR, Tremblay KD, Bartolomei MS, Schultz RM. Differential effects of culture on imprinted H19 expression in the preimplantation mouse embryo. *Biol Reprod* 2000;62:1526–35.
39. Mann MR, Lee SS, Doherty AS, Verona RI, Nolen LD, Schultz RM, et al. Selective loss of imprinting in the placenta following preimplantation development in culture. *Development* 2004;131:3727–35.
40. Market-Velker BA, Fernandes AD, Mann MR. Side-by-side comparison of five commercial media systems in a mouse model: suboptimal in vitro culture interferes with imprint maintenance. *Biol Reprod* 2010;83:938–50.
41. Fauque P, Jouannet P, Lesaffre C, Ripoche MA, Dandolo L, Vaiman D, et al. Assisted reproductive technology affects developmental kinetics, H19 imprinting control region methylation and H19 gene expression in individual mouse embryos. *BMC Dev Biol* 2007;7:116.
42. Schwarzer C, Esteves TC, Araúzo-Bravo MJ, Le Gac S, Nordhoff V, Schlatt S, et al. ART culture conditions change the probability of mouse embryo gestation through defined cellular and molecular responses. *Hum Reprod* 2012;27:2627–40.
43. Gad A, Schellander K, Hoelker M, Tesfaye D. Transcriptome profile of early mammalian embryos in response to culture environment. *Anim Reprod Sci* 2012;134:76–83.
44. Kleijkers SH, Eijssen LM, Coonen E, Derhaag JG, Mantikou E, Jonker MJ, et al. Differences in gene expression profiles between human preimplantation embryos cultured in two different IVF culture media. *Hum Reprod* 2015;30:2303–11.
45. Mantikou E, Jonker MJ, Wong KM, van Montfoort AP, de Jong M, Breit TM, et al. Factors affecting the gene expression of in vitro cultured human preimplantation embryos. *Hum Reprod* 2016;31:298–311.
46. Kimber SJ, Sneddon SF, Bloor DJ, El-Bareg AM, Hawkhead JA, Metcalfe AD, et al. Expression of genes involved in early cell fate decisions in human embryos and their regulation by growth factors. *Reproduction* 2008;135:635–47.
47. Hemkemeyer SA, Schwarzer C, Boiani M, Ehmcke J, Le Gac S, Schlatt S, et al. Effects of embryo culture media do not persist after implantation: a histological study in mice. *Hum Reprod* 2014;29:220–33.
48. Young LE, Sinclair KD, Wilmut I. Large offspring syndrome in cattle and sheep. *Rev Reprod* 1998;3:155–63.
49. Chen Z, Hagen DE, Elsik CG, Ji T, Morris CJ, Moon LE, et al. Characterization of global loss of imprinting in fetal overgrowth syndrome induced by assisted reproduction. *Proc Natl Acad Sci U S A* 2015;112:4618–23.
50. Zandstra H, Van Montfoort AP, Dumoulin JC. Does the type of culture medium used influence birthweight of children born after IVF? *Hum Reprod* 2015;30:530–42.
51. Barker DJ. The developmental origins of adult disease. *J Am Coll Nutr* 2004;23 Suppl 6:588S–595S.
52. Dumoulin JC, Land JA, Van Montfoort AP, Nelissen EC, Coonen E, Derhaag JG, et al. Effect of in vitro culture of human embryos on birthweight of newborns. *Hum Reprod* 2010;25:605–12.
53. Nelissen EC, Van Montfoort AP, Coonen E, Derhaag JG, Geraedts JP, Smits LJ, et al. Further evidence that culture media affect perinatal outcome: findings after transfer of fresh and cryopreserved embryos. *Hum Reprod* 2012;27:1966–76.

54. Kleijkers SH, van Montfoort AP, Smits LJ, Viechtbauer W, Roseboom TJ, Nelissen EC, et al. IVF culture medium affects post-natal weight in humans during the first 2 years of life. *Hum Reprod* 2014;29:661–9.
55. Lin S, Li M, Lian Y, Chen L, Liu P. No effect of embryo culture media on birthweight and length of newborns. *Hum Reprod* 2013;28:1762–7.
56. Carrasco B, Boada M, Rodríguez I, Coroleu B, Barri PN, Veiga A. Does culture medium influence offspring birth weight? *Fertil Steril* 2013;100:1283–8.
57. Eskild A, Monkerud L, Tanbo T. Birthweight and placental weight; do changes in culture media used for IVF matter? Comparisons with spontaneous pregnancies in the corresponding time periods. *Hum Reprod* 2013;28:3207–14.
58. De Vos A, Janssens R, Van de Velde H, Haentjens P, Bonduelle M, Tournaye H, et al. The type of culture medium and the duration of in vitro culture do not influence birthweight of ART singletons. *Hum Reprod* 2015;30:20–7.
59. Kleijkers SH, van Montfoort AP, Smits LJ, Coonen E, Derhaag JG, Evers JL, et al. Age of G-1 PLUS v5 embryo culture medium is inversely associated with birthweight of the newborn. *Hum Reprod* 2015;30:1352–7.
60. Zhu J, Lin S, Li M, Chen L, Lian Y, Liu P, et al. Effect of in vitro culture period on birthweight of singleton newborns. *Hum Reprod* 2014;29:448–54.
61. Zhu J, Li M, Chen L, Liu P, Qiao J. The protein source in embryo culture media influences birthweight: a comparative study between G1 v5 and G1-PLUS v5. *Hum Reprod* 2014;29:1387–92.
62. Sunde A, Brison D, Dumoulin J, Harper J, Lundin K, Magli MC, et al. Time to take human embryo culture seriously. *Hum Reprod* 2016;31:2174–82.
63. Kleijkers SH, Mantikou E, Slappendel E, Consten D, van Echten-Arends J, Wetzels AM, et al. Influence of embryo culture medium (G5 and HTF) on pregnancy and perinatal outcome after IVF: a multicenter RCT. *Hum Reprod* 2016;31:2219–30.
64. Roberts SA, Vail A. On the appropriate interpretation of evidence: the example of culture media and birth weight. Hum Reprod. 2017;32(6):1151-4.
65. Obata Y, Kono T. Maternal primary imprinting is established at a specific time for each gene throughout oocyte growth. *J Biol Chem* 2002;277:5285–9.
66. O'Doherty AM, O'Shea LC, Fair T. Bovine DNA methylation imprints are established in an oocyte size-specific manner, which are coordinated with the expression of the DNMT3 family proteins. *Biol Reprod* 2012;86:67.
67. Van der Auwera I, D'Hooghe T. Superovulation of female mice delays embryonic and fetal development. *Hum Reprod* 2001;16:1237–43.
68. Market-Velker BA, Zhang L, Magri LS, Bonvissuto AC, Mann MR. Dual effects of superovulation: loss of maternal and paternal imprinted methylation in a dose-dependent manner. *Hum Mol Genet* 2010;19:36–51.
69. Sato A, Otsu E, Negishi H, Utsunomiya T, Arima T. Aberrant DNA methylation of imprinted loci in superovulated oocytes. *Hum Reprod* 2007;22:26–35.
70. Khoueiry R, Ibala-Rhomdane S, Méry L, Blachère T, Guérin JF, Lornage J, et al. Dynamic CpG methylation of the *KCNQ1OT1* gene during maturation of human oocytes. *J Med Genet* 2008;45:583–8.
71. Chang AS, Moley KH, Wangler M, Feinberg AP, Debaun MR. Association between Beckwith-Wiedemann syndrome and assisted reproductive technology: a case series of 19 patients. *Fertil Steril* 2005;83:349–54.
72. Stouder C, Deutsch S, Paoloni-Giacobino A. Superovulation in mice alters the methylation pattern of imprinted genes in the sperm of the offspring. *Reprod Toxicol* 2009;28:536–41.
73. Fortier AL, Lopes FL, Darricarrère N, Martel J, Trasler JM. Superovulation alters the expression of imprinted genes in the midgestation mouse placenta. *Hum Mol Genet* 2008;17:1653–65.

74. Nakamura T, Arai Y, Umehara H, Masuhara M, Kimura T, Taniguchi H, et al. PGC7/Stella protects against DNA demethylation in early embryogenesis. *Nat Cell Biol* 2007;9:64–71.
75. Denomme MM, Zhang L, Mann MR. Embryonic imprinting perturbations do not originate from superovulation-induced defects in DNA methylation acquisition. *Fertil Steril* 2011;96:734–8.e2.
76. Huffman SR, Pak Y, Rivera RM. Superovulation induces alterations in the epigenome of zygotes, and results in differences in gene expression at the blastocyst stage in mice. *Mol Reprod Dev* 2015;82:207–17.
77. Santos F, Hyslop L, Stojkovic P, Leary C, Murdoch A, Reik W, et al. Evaluation of epigenetic marks in human embryos derived from IVF and ICSI. *Hum Reprod* 2010;25:2387–95.
78. Geuns E, De Rycke M, Van Steirteghem A, Liebaers I. Methylation imprints of the imprint control region of the *SNRPN*-gene in human gametes and preimplantation embryos. *Hum Mol Genet* 2003;12:2873–9.
79. Shi X, Chen S, Zheng H, Wang L, Wu Y. Abnormal DNA methylation of imprinted loci in human preimplantation embryos. *Reprod Sci* 2014;21:978–83.
80. Chen SL, Shi XY, Zheng HY, Wu FR, Luo C. Aberrant DNA methylation of imprinted H19 gene in human preimplantation embryos. *Fertil Steril* 2010;94:2356–8.e1.
81. Huntriss J, Picton HM. Epigenetic consequences of assisted reproduction and infertility on the human preimplantation embryo. *Hum Fertil (Camb)* 2008;11:85–94.
82. Chen Z, Robbins KM, Wells KD, Rivera RM. Large offspring syndrome: a bovine model for the human loss-of-imprinting overgrowth syndrome Beckwith-Wiedemann. *Epigenetics* 2013;8:591–601.
83. White CR, Denomme MM, Tekpetey FR, Feyles V, Power SG, Mann MR. High frequency of imprinted methylation errors in human preimplantation embryos. *Sci Rep* 2015;5:17311.
84. Khoueiry R, Ibala-Romdhane S, Al-Khtib M, Blachère T, Lornage J, Guérin JF, et al. Abnormal methylation of KCNQ1OT1 and differential methylation of H19 imprinting control regions in human ICSI embryos. *Zygote* 2013;21:129–38.
85. Ibala-Romdhane S, Al-Khtib M, Khoueiry R, Blachère T, Guérin JF, Lefèvre A. Analysis of H19 methylation in control and abnormal human embryos, sperm and oocytes. *Eur J Hum Genet* 2011;19:1138–43.
86. Huntriss JD, Hemmings KE, Hinkins M, Rutherford AJ, Sturmey RG, Elder K, et al. Variable imprinting of the *MEST* gene in human preimplantation embryos. *Eur J Hum Genet* 2013;21:40–7.
87. Stuppia L, Franzago M, Ballerini P, Gatta V, Antonucci I. Epigenetics and male reproduction: the consequences of paternal lifestyle on fertility, embryo development, and children lifetime health. *Clin Epigenetics* 2015;7:120.
88. Boissonnas CC, Jouannet P, Jammes H. Epigenetic disorders and male subfertility. *Fertil Steril* 2013;99:624–31.
89. Hotaling J, Carrell DT. Clinical genetic testing for male factor infertility: current applications and future directions. *Andrology* 2014;2:339–50.
90. Aston KI, Carrell DT. Prospects for clinically relevant epigenetic tests in the andrology laboratory. *Asian J Androl* 2014;16:782.
91. Kläver R, Gromoll J. Bringing epigenetics into the diagnostics of the andrology laboratory: challenges and perspectives. *Asian J Androl* 2014;16:669–74.
92. Kobayashi H, Hiura H, John RM, Sato A, Otsu E, Kobayashi N, et al. DNA methylation errors at imprinted loci after assisted conception originate in the parental sperm. *Eur J Hum Genet* 2009;17:1582–91.
93. Song S, Ghosh J, Mainigi M, Turan N, Weinerman R, Truongcao M, et al. DNA methylation differences between in vitro- and in vivo-conceived children are associated with ART procedures rather than infertility. *Clin Epigenetics* 2015;7:41.

94. Jenkins TG, Aston KI, Pflueger C, Cairns BR, Carrell DT. Age-associated sperm DNA methylation alterations: possible implications in offspring disease susceptibility. *PLoS Genet* 2014;10:e1004458.
95. de Castro Barbosa T, Ingerslev LR, Alm PS, Versteyhe S, Massart J, Rasmussen M, et al. High-fat diet reprograms the epigenome of rat spermatozoa and transgenerationally affects metabolism of the offspring. *Mol Metab* 2016;5:184–97.
96. Ge ZJ, Liang QX, Hou Y, Han ZM, Schatten H, Sun QY, et al. Maternal obesity and diabetes may cause DNA methylation alteration in the spermatozoa of offspring in mice. *Reprod Biol Endocrinol* 2014;12:29.
97. Soubry A, Hoyo C, Jirtle RL, Murphy SK. A paternal environmental legacy: evidence for epigenetic inheritance through the male germ line. *BioEssays* 2014;36:359–71.
98. Shea JM, Serra RW, Carone BR, Shulha HP, Kucukural A, Ziller MJ, et al. Genetic and epigenetic variation, but not diet, shape the sperm methylome. *Dev Cell* 2015;35:750–8.
99. Batcheller A, Cardozo E, Maguire M, DeCherney AH, Segars JH. Are there subtle genome-wide epigenetic alterations in normal offspring conceived by assisted reproductive technologies? *Fertil Steril* 2011;96:1306–11.
100. Whitelaw N, Bhattacharya S, Hoad G, Horgan GW, Hamilton M, Haggarty P. Epigenetic status in the offspring of spontaneous and assisted conception. *Hum Reprod* 2014;29:1452–8.
101. Melamed N, Choufani S, Wilkins-Haug LE, Koren G, Weksberg R. Comparison of genome-wide and gene-specific DNA methylation between ART and naturally conceived pregnancies. *Epigenetics* 2015;10:474–83.
102. Cetin I, Cozzi V, Antonazzo P. Fetal development after assisted reproduction—a review. *Placenta* 2003;24 Suppl B:S104–13.
103. Källén B, Finnström O, Lindam A, Nilsson E, Nygren KG, Otterblad PO. Congenital malformations in infants born after in vitro fertilization in Sweden. *Birth Defects Res A Clin Mol Teratol* 2010;88:137–43.
104. Hart R, Norman RJ. The longer-term health outcomes for children born as a result of IVF treatment: Part I–General health outcomes. *Hum Reprod Update* 2013;19:232–43.
105. Scherrer U, Rimoldi SF, Rexhaj E, Stuber T, Duplain H, Garcin S, et al. Systemic and pulmonary vascular dysfunction in children conceived by assisted reproductive technologies. *Circulation* 2012;125:1890–6.
106. von Arx R, Allemann Y, Sartori C, Rexhaj E, Cerny D, de Marchi SF, et al. Right ventricular dysfunction in children and adolescents conceived by assisted reproductive technologies. *J Appl Physiol (1985)* 2015;118:1200–6.
107. Valenzuela-Alcaraz B, Crispi F, Bijnens B, Cruz-Lemini M, Creus M, Sitges M, et al. Assisted reproductive technologies are associated with cardiovascular remodeling in utero that persists postnatally. *Circulation* 2013;128:1442–50.
108. Rexhaj E, Paoloni-Giacobino A, Rimoldi SF, Fuster DG, Anderegg M, Somm E, et al. Mice generated by in vitro fertilization exhibit vascular dysfunction and shortened life span. *J Clin Invest* 2013;123:5052–60.
109. Chen M, Wu L, Zhao J, Wu F, Davies MJ, Wittert GA, et al. Altered glucose metabolism in mouse and humans conceived by IVF. *Diabetes* 2014;63:3189–98.
110. Ceelen M, van Weissenbruch MM, Roos JC, Vermeiden JP, van Leeuwen FE, Delemarre-van de Waal HA. Body composition in children and adolescents born after in vitro fertilization or spontaneous conception. *J Clin Endocrinol Metab* 2007;92:3417–23.
111. Ceelen M, van Weissenbruch MM, Vermeiden JP, van Leeuwen FE, Delemarre-van de Waal HA. Cardiometabolic differences in children born after in vitro fertilization: follow-up study. *J Clin Endocrinol Metab* 2008;93:1682–8.
112. Sakka SD, Loutradis D, Kanaka-Gantenbein C, Margeli A, Papastamataki M, Papassotiriou I, et al. Absence of insulin resistance and low-grade inflammation despite early metabolic syndrome manifestations in children born after in vitro fertilization. *Fertil Steril* 2010;94:1693–9.

113. Sakka SD, Margeli A, Loutradis D, Chrousos GP, Papassotiriou I, Kanaka-Gantenbein C. Gender dimorphic increase in RBP-4 and NGAL in children born after IVF: an epigenetic phenomenon? *Eur J Clin Invest* 2013;43:439–48.
114. Rimoldi SF, Sartori C, Rexhaj E, Cerny D, Von Arx R, Soria R, et al. Vascular dysfunction in children conceived by assisted reproductive technologies: underlying mechanisms and future implications. *Swiss Med Wkly* 2014;144:w13973.
115. Kaelin WG Jr, McKnight SL. Influence of metabolism on epigenetics and disease. *Cell* 2013;153:56–69.
116. Steegers-Theunissen RP, Twigt J, Pestinger V, Sinclair KD. The periconceptional period, reproduction and long-term health of offspring: the importance of one-carbon metabolism. *Hum Reprod Update* 2013;19:640–55.
117. Refsum H, Nurk E, Smith AD, Ueland PM, Gjesdal CG, Bjelland I, et al. The Hordaland Homocysteine Study: a community-based study of homocysteine, its determinants, and associations with disease. *J Nutr* 2006;136 Suppl 6:1731S–1740S.
118. Drake AJ, O'Shaughnessy PJ, Bhattacharya S, Monteiro A, Kerrigan D, Goetz S, et al. In utero exposure to cigarette chemicals induces sex-specific disruption of one-carbon metabolism and DNA methylation in the human fetal liver. *BMC Med* 2015;13:18.
119. Haavaldsen C, Tanbo T, Eskild A. Placental weight in singleton pregnancies with and without assisted reproductive technology: a population study of 536,567 pregnancies. *Hum Reprod* 2012;27:576–82.
120. Nelissen EC, Dumoulin JC, Busato F, Ponger L, Eijssen LM, Evers JL, et al. Altered gene expression in human placentas after IVF/ICSI. *Hum Reprod* 2014;29:2821–31.
121. Vincent RN, Gooding LD, Louie K, Chan Wong E, Ma S. Altered DNA methylation and expression of PLAGL1 in cord blood from assisted reproductive technology pregnancies compared with natural conceptions. *Fertil Steril* 2016;106:739–48.
122. Monk D. Genomic imprinting in the human placenta. *Am J Obstet Gynecol* 2015;213 Suppl 4:S152–62.
123. Chen S, Sun FZ, Huang X, Wang X, Tang N, Zhu B, et al. Assisted reproduction causes placental maldevelopment and dysfunction linked to reduced fetal weight in mice. *Sci Rep* 2015;5:10596.
124. Li B, Chen S, Tang N, Xiao X, Huang J, Jiang F, et al. Assisted reproduction causes reduced fetal growth associated with downregulation of paternally expressed imprinted genes that enhance fetal growth in mice. *Biol Reprod* 2016;94:45.
125. Bloise E, Lin W, Liu X, Simbulan R, Kolahi KS, Petraglia F, et al. Impaired placental nutrient transport in mice generated by in vitro fertilization. *Endocrinology* 2012;153:3457–67.
126. de Waal E, Vrooman LA, Fischer E, Ord T, Mainigi MA, Coutifaris C, et al. The cumulative effect of assisted reproduction procedures on placental development and epigenetic perturbations in a mouse model. *Hum Mol Genet* 2015;24:6975–85.
127. Choux C, Carmignac V, Bruno C, Sagot P, Vaiman D, Fauque P. The placenta: phenotypic and epigenetic modifications induced by assisted reproductive technologies throughout pregnancy. *Clin Epigenetics* 2015;7:87.

This Scientific Impact Paper was produced on behalf of the Royal College of Obstetricians and Gynaecologists by:
Dr J Huntriss, Division of Reproduction and Early Development, Leeds Institute of Cardiovascular and Metabolic Medicine; Professor AH Balen FRCOG, Leeds; Professor KD Sinclair, School of Biosciences, University of Nottingham; Professor DR Brison, Department of Reproductive Medicine, Manchester University NHS Foundation Trust, Manchester Academic Health Sciences Centre; and Professor HM Picton, Division of Reproduction and Early Development, Leeds Institute of Cardiovascular and Metabolic Medicine

and peer reviewed by:
Dr A Drake MBBS PhD FRCPCH, Centre for Cardiovascular Science, The University of Edinburgh; Mr DI Fraser FRCOG, Norwich; and RCOG Women's Network.

The Scientific Advisory Committee lead reviewer was: Professor H Leese FRCOG, Hull.

The chair of the Scientific Advisory Committee was: Dr S Ghaem-Maghami MRCOG, London.

All RCOG guidance developers are asked to declare any conflicts of interest. A statement summarising any conflicts of interest for this Scientific Impact Paper is available from: https://www.rcog.org.uk/en/guidelines-research-services/guidelines/sipXX/.

The final version is the responsibility of the Scientific Advisory Committee of the RCOG.

The paper will be considered for update 3 years after publication, with an intermediate assessment of the need to update 2 years after publication.