Title

Comparative intrauterine development and placental function of ART concepti: implications for human reproductive medicine and animal breeding

Journal

Human Reproduction Update, 20(6)

ISSN

1355-4786

Authors

Bloise, Enrrico
Feuer, Sky K
Rinaudo, Paolo F

Publication Date

2014-11-01

DOI

10.1093/humupd/dmu032

Peer reviewed

Human Reproduction Update, Vol.20, No.6 pp. 822–839, 2014
Advanced Access publication on June 19, 2014 doi:10.1093/humupd/dmu032

human reproduction update

Comparative intrauterine development and placental function of ART concepti: implications for human reproductive medicine and animal breeding

Enrrico Bloise[1], Sky K. Feuer[2], and Paolo F. Rinaudo[2,*]

[1]Laboratory of Molecular Endocrinology, Carlos Chagas Filho Biophysics Institute, Federal University of Rio de Janeiro, Rio de Janeiro, RJ, Brazil
[2]Department of Obstetrics, Gynecology and Reproductive Sciences, University of California San Francisco, San Francisco, CA, USA

*Correspondence address. E-mail: rinaudop@obgyn.ucsf.edu

Submitted on April 16, 2014; resubmitted on May 21, 2014; accepted on May 28, 2014

TABLE OF CONTENTS

BACKGROUND: The number of children conceived using assisted reproductive technologies (ART) has reached >5 million worldwide and continues to increase. Although the great majority of ART children are healthy, many reports suggest a forthcoming risk of metabolic complications, which is further supported by the Developmental Origins of Health and Disease hypothesis of suboptimal embryo/fetal conditions predisposing adult cardiometabolic pathologies. Accumulating evidence suggests that fetal and placental growth kinetics are important features predicting post-natal health, but the relationship between ART and intrauterine growth has not been systematically reviewed.

METHODS: Relevant studies describing fetoplacental intrauterine phenotypes of concepti generated by *in vitro* fertilization (IVF), intracytoplasmic sperm injection (ICSI) and somatic cell nuclear transfer (SCNT) in the mouse, bovine and human were comprehensively researched using PubMed and Google Scholar. Intrauterine growth plots were created from tabular formatted data available in selected reports.

RESULTS: ART pregnancies display minor but noticeable alterations in fetal and placental growth curves across mammalian species. In all species, there is evidence of fetal growth restriction in the earlier stages of pregnancy, followed by significant increases in placental size and accelerated fetal growth toward the end of gestation. However, there is a species-specific effect of ART on birthweights, that additionally vary in a culture condition-, strain-, and/or stage at transfer-specific manner. We discuss the potential mechanisms that underlie these changes, and how they are affected by specific components of ART procedures.

CONCLUSIONS: ART may promote measurable alterations to intrauterine growth trajectory and placental function. Key findings include evidence that birthweight is not a reliable marker of fetal stress, and that increases in embryo manipulation result in more deviant fetal growth curves. Because growth kinetics in early life are particularly relevant to adult metabolic physiology, we advise more rigorous assessment of fetal growth and

placental function in human ART pregnancies, as well as continued follow-up of ART offspring throughout post-natal life. Finally, strategies to minimize embryo manipulations should be adopted whenever possible.

Key words: assisted reproductive technology / IVF / Developmental Origins of Health and Disease / fetal and placental growth

Introduction

The Developmental Origins of Health and Disease (DOHaD) hypothesis states that an adverse maternal environment can affect early development by inducing permanent changes in physiology and metabolism, leading to increased disease risk in adulthood (Fig. 1) (Barker, 2007). This is especially relevant to the field of assisted reproductive technology (ART). Since the first successful birth from *in vitro* fertilization (IVF) (Fig. 2) in 1978, the number of children born through ART has increased to over 5 million worldwide. In the Western world, an estimated 1% of children born annually are conceived by ART (ICMART, 2012). The great majority of these children are healthy (Davies *et al.*, 2012), although several reports have demonstrated the potential for adverse perinatal conditions in ART pregnancies (Templeton, 2000; Hansen *et al.*, 2013). For example, there is an increased risk for several pregnancy complications, including pre-eclampsia (Chen *et al.*, 2009), placenta praevia (Romundstad *et al.*, 2006), placenta accreta (Esh-Broder *et al.*, 2011), abnormal placental growth, perinatal mortality, preterm delivery, and most importantly, low birthweight (Schieve *et al.*, 2002; Helmerhorst *et al.*, 2004; Jackson *et al.*, 2004; Ceelen *et al.*, 2008; Rinaudo and Lamb, 2008; Haavaldsen *et al.*, 2012). Given the importance of intrauterine environment for both fetal growth and adult health—as articulated by the DOHaD hypothesis—the increases in pregnancy complications following ART are of particular concern.

In the following paragraphs, evidence from murine, bovine and finally human data is used to comprehensively describe the intrauterine environment and related biological alterations of ART concepti. Because it is problematic to tease out whether ART-associated adverse outcomes are secondary to the ART procedures or to parental factors, animal models have been especially useful in elucidating intrauterine growth and placentation changes associated with ART. Mouse and bovine models of IVF exhibit intrauterine growth restriction (IUGR) in early pregnancy, followed by accelerated fetal growth rates from mid- to late gestation, correlated with increased placental growth. Changes in fetal and placental growth phenotype are additionally influenced by ART procedure and preimplantation embryo culture conditions, suggesting that more severe embryo manipulations can induce greater abberancies in conceptus phenotype. Bovine studies additionally show a very clear and unique phenotype associated with ART, the so-called 'Large Offspring Syndrome' (LOS) (Sinclair *et al.*, 2000; Farin *et al.*, 2006); a condition also observed in the sheep (Young *et al.*, 1998). It is important to emphasize the existence of morphological, temporal and metabolic differences in early embryo development among mammalian species (Prather and First, 1988; Richardson *et al.*, 1997). Therefore, caution is advised when extrapolating the significance of these studies to human ART, as animal models may provide imprecise views of the demands of human development.

Methods

To systematically review the intrauterine phenotype of embryos and fetuses generated by IVF, intracytoplasmic sperm injection (ICSI) and somatic cell nuclear transfer (SCNT), PubMed and Google Scholar were used to identify relevant studies in the mouse, bovine and human. The search strategy comprised a combination of the following terms: IVF, ICSI, SCNT, mouse, bovine/ruminant, human, endometrium, maternal recognition of pregnancy, embryo, fetal growth, placenta, ultrasound, oxygen tension, transcriptome, proteomics, abortion and epigenetics. The intrauterine growth trajectory plots were created from data available in the format of tables in selected reports (cited when pertinent), and statistical significance is displayed as described in the original reports. Only literature written in English was included, without restriction on year of publication. We discuss the current knowledge of ART-induced effects on fetal intrauterine growth trajectory and placental growth, function and physiology, including potential molecular mechanisms underlying the altered phenotypes observed following assisted conception.

ART and Recognition of Pregnancy and Miscarriage

Much attention has been devoted toward minimizing low pregnancy rates, fetal demise and other adverse perinatal outcomes in ART pregnancies. However, it is difficult to compare pregnancy rates and the incidence of miscarriage in IVF versus spontaneously conceived pregnancies, making animal models particularly valuable for these analyses (Table I). In the mouse, IVF is linked with increased abortion rates by embryonic day E12.5 (Delle Piane *et al.*, 2010), a gestational age marked by both the establishment of the chorioallantoic placenta and the onset of fetal dependence upon placental uptake of nutrients from maternal circulation (Malassiné *et al.*, 2003). This study described significantly increased abortion rates for IVF embryos grown under suboptimal conditions (26.89%, Whitten's culture medium and 20% oxygen tension), compared with *in vivo* embryos (7.09%) or *in vivo*-generated blastocysts transferred to surrogate dams (flushed blastocyst control group, 7.28% abortion rates). IVF embryos produced in optimized conditions (using K simplex optimized medium (KSOM) with amino acids and 5% oxygen) also had higher abortion rates (17.00%), although this was not significantly different from *in vivo* or flushed blastocyst controls (Delle Piane *et al.*, 2010). Interestingly, implantation rates were not different among the four groups. Hemkemeyer *et al.* similarly did not find significant implantation changes between ART mice cultured in different conditions (Hemkemeyer *et al.*, 2014). This indicates that preimplantation culture conditions can affect mouse embryo survival *after* the implantation period, which may be related to placentation processes or failed maternal recognition of pregnancy.

Bovine models also demonstrate significant loss of *in vitro*-generated pregnancies, predominantly during the earliest stages of gestation. In fact, the greatest risk of spontaneous abortion occurs during embryogenesis (extending from conception to the completion of organogenesis, Day 1 to Day 42) rather than the fetal period (completion of organogenesis to parturition, Day 42 to Day 280) or neonatal period (parturition through Day 28 of extra-uterine life) (Farin *et al.*, 2001, 2006). A 55% loss rate has been described for *in vitro*-produced (IVP) embryos at term (Kruip and Daas, 1997) compared with the *in vivo* incidence of

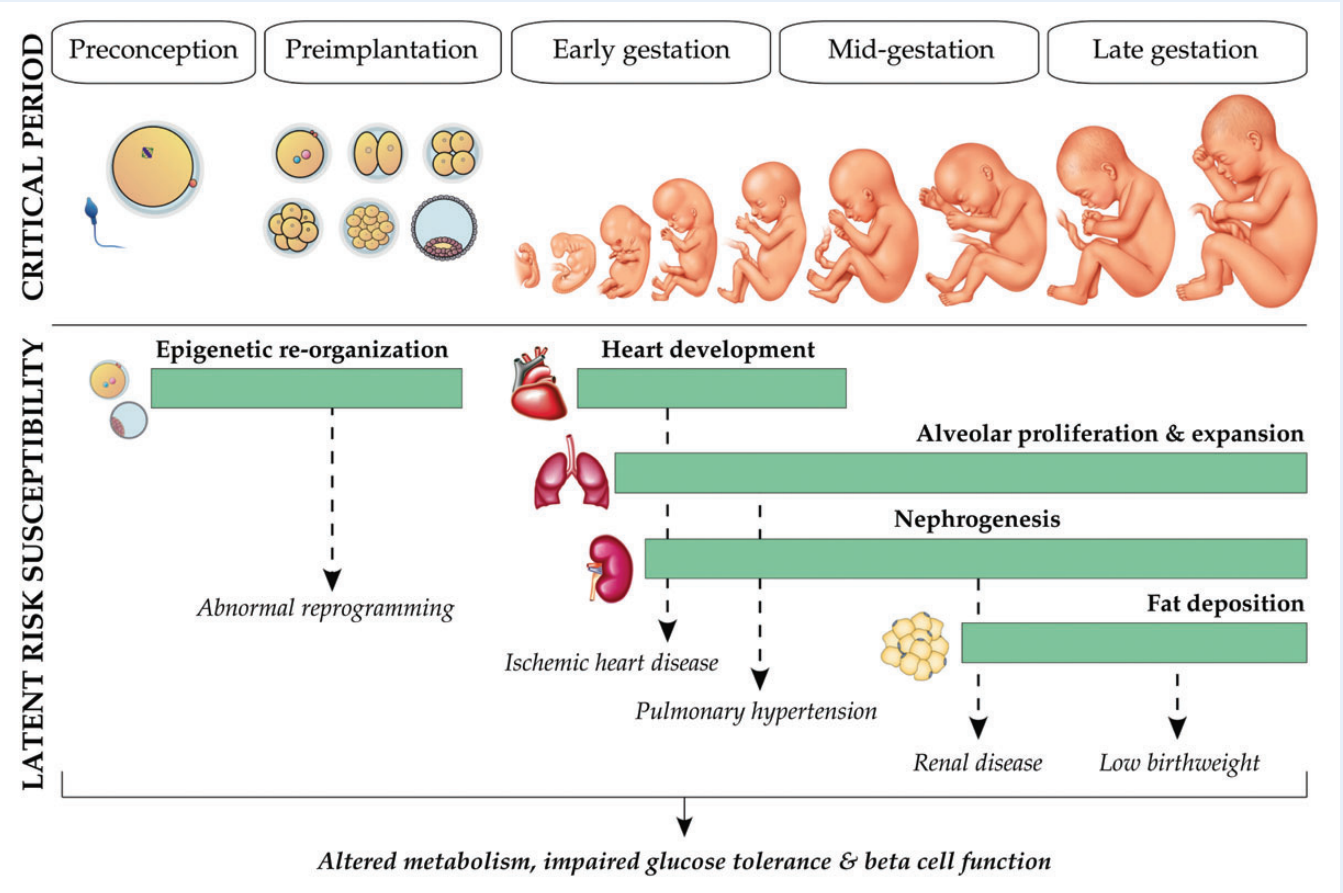

Figure 1 Critical period of developmental sensitivity. Schematic illustrating the windows of human pregnancy most susceptible for influencing the development of components of metabolic syndrome later in life. Metabolic stress, including defects in glucose handling, is a common outcome of stress *in utero* and is less dependent on the specific timing of the stress occurrence. In addition to increased vulnerability during organogenesis, the preimplantation period is a time of extensive epigenetic remodeling and therefore may be particularly susceptible to stress.

<5% (King *et al.*, 1985; Farin *et al.*, 2006); a separate study reported a 45% loss rate at Day 14 (McMillan *et al.*, 1997), consistent with the bovine maternal recognition of pregnancy window (Mamo *et al.*, 2011). SCNT-derived pregnancies are also susceptible to embryo/fetal demise, with reported cumulative embryonic loss reaching 25% around gestational Day 30, increasing to 50% by Day 39, and totaling ~75% by the end of gestation (Pace *et al.*, 2002). Similar cumulative percentages of SCNT pregnancy losses have been independently corroborated (Watanabe and Nagai, 2011). Importantly, increased embryonic and fetal loss ratios vary according to culture media, and ART protocol used (described below) (Farin *et al.*, 2006).

Overall, whether spontaneous abortion is higher in human ART pregnancies is not well established (Tummers *et al.*, 2003), and there is unfortunately a lack of comprehensive studies evaluating the relationship between ART and abortion rates which control for maternal age, BMI, and ethnicity, among other factors. In the general population, spontaneous abortions occur in an estimated 12–24% of reproductive-aged women conceiving naturally (Jurkovic *et al.*, 2013). The Canadian ART Register reported pregnancy losses of 14.9–15.4% in ART conceptions (IVF and ICSI) in the year 2006–2007 (Gunby *et al.*, 2010, 2011). A retrospective study of 1597 clinical pregnancies conceived by IVF and ICSI showed a 21.7% global incidence of spontaneous abortion in singleton ART pregnancies (Tummers *et al.*, 2003), and another group reported a slightly increased risk of spontaneous abortion for ART pregnancies over natural conception (relative risk of 1.20, 95% Confidence Interval 1.03–1.46) (Wang *et al.*, 2004). Aytoz and colleagues evaluated the obstetric outcome for ART pregnancies derived from fresh or cryopreserved embryos generated by IVF or ICSI. Abortion rates were comparable between fresh and frozen IVF embryos (13.1%), whereas freshly transferred ICSI embryos showed increased abortion rates (18.6%) and frozen-thawed ICSI embryos the highest (26.0%, $P < 0.05$) (Aytoz *et al.*, 1999). Although certain ART conditions are linked with significant increases in miscarriage, overall these numbers are similar to the estimated rates for the naturally conceiving population and an effect of ART on abortion rate is not definitive (Jurkovic *et al.*, 2013).

Chromosomal abnormalities in developing embryos are the most well-described cause for spontaneous abortion (Brown, 2008), and there is little evidence linking ART procedures with chromosomal irregularities in developing concepti. Analysis comparing cytogenetic profiles of abortuses from 133 ART patients (IVF and ICSI) to 144 samples from naturally conceiving subfertile couples revealed similar levels of karyotype defects between ART (63.2%) and spontaneously conceived

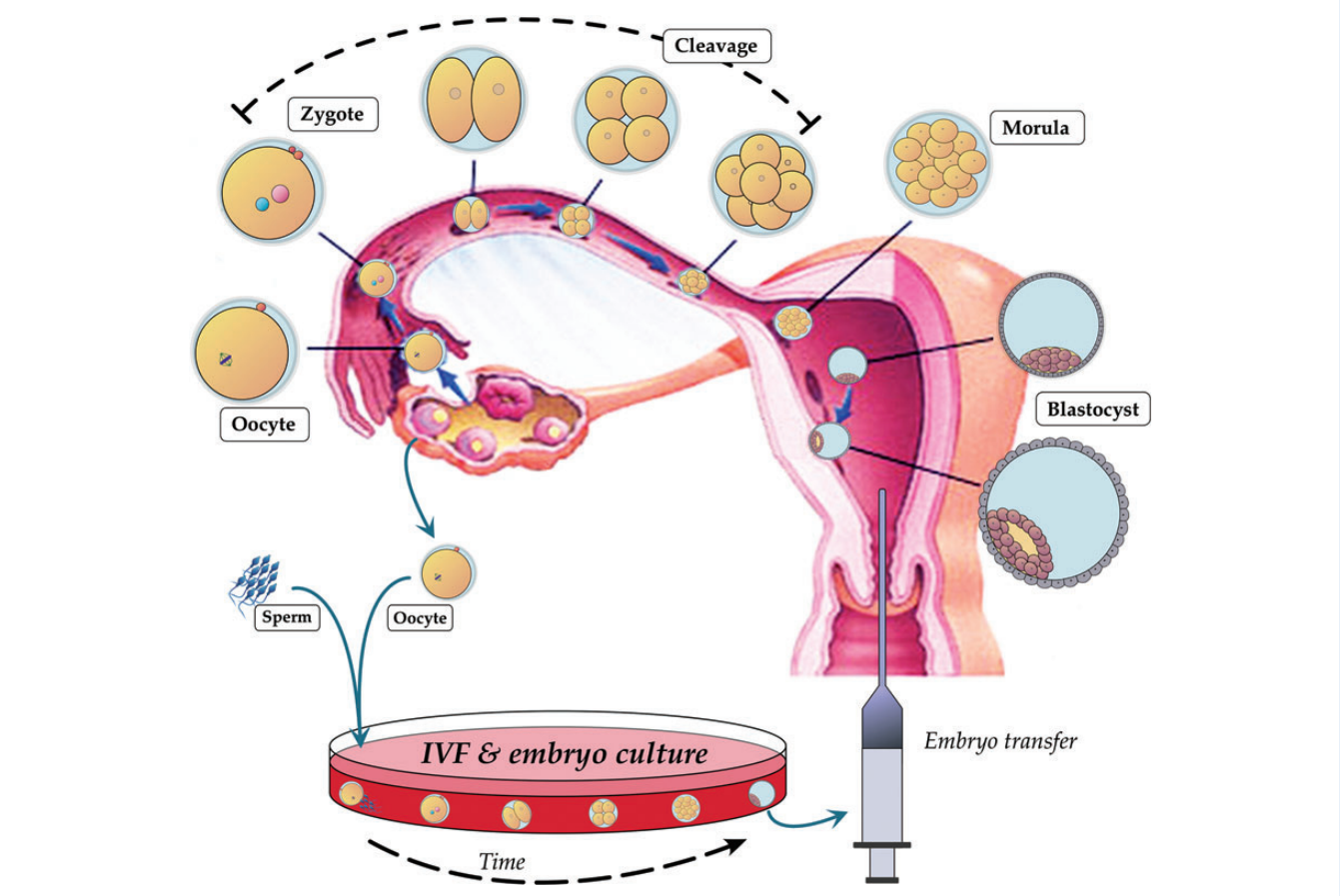

Figure 2 Preimplantation development and IVF. Overview of the stages of preimplantation embryo development from ovulation and fertilization of the oocyte in the oviduct, through cleavage divisions and embryo polarization, to blastocyst formation and implantation into the uterus. *In vitro*, gametes are isolated for co-incubation, and fertilized zygotes are cultured 3–5 days prior to transfer back into the uterus.

Table I Fetal loss/miscarriage rates in assisted reproductive technology (ART) concepti.

Species	Method of conception	Medium	Gestational age	Miscarriage, abortion rates % (~)	References
Mouse	IVF	K_{AA}	E12.5	17	Delle Piane *et al.* (2010)
	IVF	Whitten's	E12.5	27	
	In vivo-flushed blastocyst	–	E12.5	7	
	In vivo	–	E12.5	7	
Bovine	IVF	Medium-199	Term	55	Kruip and Daas (1997)
	SCNT	–	Term	75	Pace *et al.* (2002) and Watanabe and Nagai (2011)
	In vivo	–	Term	5	King *et al.* (1985) and Farin *et al.* (2006)
Human	IVF/ICSI	–	–	15/22	Gunby *et al.* (2010), Gunby *et al.* (2011) and Tummers *et al.* (2003)
	Freshly transferred ICSI	–	–	19	Aytoz *et al.* (1999)
	Frozen-thawed transferred ICSI	–	–	26	Aytoz *et al.* (1999)
	Spontaneous conception	–	–	12-24	Jurkovic *et al.* (2013)

E, embryonic day; SCNT, somatic cell nuclear transfer.

miscarriages (71.5%). Furthermore, the incidence of aneuploidy did not differ between IVF (54.5%) and ICSI (61.5%) (Bettio *et al.*, 2008). Conversely, other groups have reported both significant increases or no changes in the number of cytogenetic anomalies for ICSI versus IVF miscarriages, although these studies are caveated by small sample size (Bettio *et al.*, 2008).

In summary, the incidence of miscarriage does not appear noticeably dissimilar between naturally conceived and ART pregnancies; however more studies are warranted to confirm this.

Effects of ART on Intrauterine Growth Trajectory

Fetal needs across pregnancy are met by the placenta, a temporary endocrine gland that transfers oxygen and nutrients from the mother to the fetus. Placental interface structure and function is adjusted by the fetus throughout pregnancy, in response to changes in maternal ecology and resource availability (Rutherford *et al*, 2009). These adaptations are required to provide the best developmental scenario possible to properly coordinate fetal growth and development. Indeed, placental defects are a frequent cause of abnormal fetal development and pregnancy failure (Hill *et al.*, 2000; Norwitz 2006). Therefore, placental developmental competence is integrally linked to developmental stress of the fetus.

Numerous reports using a variety of models have highlighted an effect of embryo manipulation on subsequent fetal growth and placentation. Specifically, there is growing evidence that many ART procedures are linked with fetal growth restriction in early to mid-pregnancy, followed by significant increases in placental size and accelerated fetal growth toward the end of gestation (Fig. 3). To this end, a particularly valuable marker used to assess developmental stress, placental function and nutrient transport efficiency is the fetal:placental (F:P) weight ratio (Desforges and Sibley, 2010). There is evidence that alterations to F:P ratio during intrauterine life, as well as post-natal rapid or 'catch-up' growth velocity, can predispose cardiometabolic complications in adulthood, indicating the importance of closely following the changes in fetal developmental kinetics associated with ART pregnancies (Barker *et al.*, 1992; Phipps *et al.*, 1993; Martyn *et al.*, 1996; Barker 2007). Dissecting the trajectory of intrauterine growth (both placental and fetal patterns) is therefore useful for understanding mechanisms of reprogramming.

Studies in rodents indicate ART embryos initially implant with significantly fewer inner cell mass (ICM) cells (Giritharan *et al.*, 2007, 2010), and remain smaller than control fetuses throughout early, mid-, and late pregnancy (Delle Piane *et al.*, 2010; Bloise *et al.*, 2012). An investigation of fetal growth using CF1 x B6D2F1/J outbred embryos generated by IVF in optimal culture conditions (KSOM medium supplemented with amino acids and 5% oxygen) and transferred to surrogate dams at the blastocyst stage reported significantly lower fetal weights at embryonic days E12.5, E15.5 and E18.5 compared with control fetuses derived *in vivo* but also transferred to pseudo-pregnant recipients at the blastocyst stage (Delle Piane *et al.*, 2010; Bloise *et al.*, 2012). Growth restriction was exacerbated when embryos were generated using a suboptimal medium, Whitten's medium (Delle Piane *et al.*, 2010), indicating that fetal weight after ART is highly dependent upon culture medium composition (Fig. 4). Interestingly, birthweights were statistically indistinguishable between both optimal and suboptimal IVF cohorts and the control mice (Bloise *et al.*, 2012), demonstrating that ART mouse embryos undergo an intrauterine catch-up growth to reach normal birthweight (Bloise *et al.*, 2012). A different study showed increased birthweight in IVF mouse concepti following transfer of 2-cell IVF C57Bl/6J embryos previously fertilized and cultured 24 h in human tubal fluid (HTF) (Le *et al.*, 2013). The birthweight disparities in different studies could be attributed to the variations in culture media, mouse strain and/or stage of embryo transfer.

The unique fetal growth velocities observed in ART models may be related to adaptations in placental phenotype. In the IVF concepti described above, placentae became progressively larger and near term were 25.4% heavier than controls (Delle Piane *et al.*, 2010; Bloise *et al.*, 2012). These embryos were further characterized by a significantly decreased F:P weight ratio at E12.5, E15.5 and E18.5 (Fig. 3). This indicates that placental phenotype is highly responsive to conditions *in vitro*. Placentomegaly has additionally been observed at E18.5 for both IVF and ICSI inbred B6D2F1 embryos cultured in CZB medium and transferred at the 2-cell stage (Collier *et al.*, 2009). Therefore, placental enlargement is a common outcome in late pregnancy following ART in mice—regardless of the form of ART applied—and a reasonable mechanism by which IVF embryos can accelerate intrauterine growth to reach normal birthweights. Interestingly, no major differences in fetal or placental growth occurred with transfer of blastocysts derived from zygotes flushed out of the uterus after natural mating (Hemkemeyer *et al.*, 2014). The fact that zygote culture is not associated with abnormal intrauterine growth, whereas IVF is, would indicate that the actual fertilization process and/or initial 24 h period *in vitro* is the sensitive window responsible for altering development, although this hypothesis requires further evaluation.

Fetal growth restriction has also been associated with ART in ruminant models, with IVF-conceived embryos, fetuses, placentae, and offspring differing greatly in morphology and developmental competence compared with those derived *in vivo* (Drost, 2007). Length of ovine IVF concepti was statistically reduced on E22 (Grazul-Bilska *et al.*, 2013) and E24 (Ptak *et al.*, 2013) (of a ~147 day gestation). Similarly, in the bovine, fetal ultrasonographic morphometry detected reduced size for *in vitro*-produced concepti between E37 and E58, but these calves exhibited higher birthweights than *in vivo* controls at the end of the 285-day gestation period (Bertolini *et al.*, 2002). Notably, IVF F:P ratios differed according to the culture medium used at E70 (Miles *et al.*, 2005), consistent with the mid-pregnancy findings in mouse models (Delle Piane *et al.*, 2010). This reflects a biphasic growth velocity in which the first trimester is marked by limited fetal growth, and subsequently accelerates to yield higher birthweight calves (e.g. the bovine LOS phenotype) (Fig. 3).

In humans, low birthweight is a well-described complication of ART pregnancy (Walker *et al.*, 2000; Rinaudo and Lamb, 2008; Haavaldsen *et al.*, 2012), although few studies have investigated placental size. An extensive analysis in the Norwegian population compared placental and newborn weights in 536 567 singleton pregnancies, including 4557 conceived by IVF, 3192 through ICSI, and 88 pregnancies by the combined use of IVF and ICSI or other unspecified ART procedures (Haavaldsen *et al.*, 2012). As previously reported, ART singletons had significantly lower birthweights than spontaneously conceived (SC) controls (3451.0 g compared with 3560.6 g, respectively), but were additionally characterized by significantly increased placentae (678.9 g versus 673.0 g) and F:P ratio (0.20 for ART versus 0.19 in SC). Changes in birthweight, placental size and F:P weight ratio remained significant regardless of the form of ART, as well as through subsequent adjustment for length of gestation, sex, parity, maternal age and pregnancy complications. It is however worth noting that the statistical significance of these findings may be related to the appreciably large size of the control group, as the impact of ART on birthweight and placental size totaled just 3 and

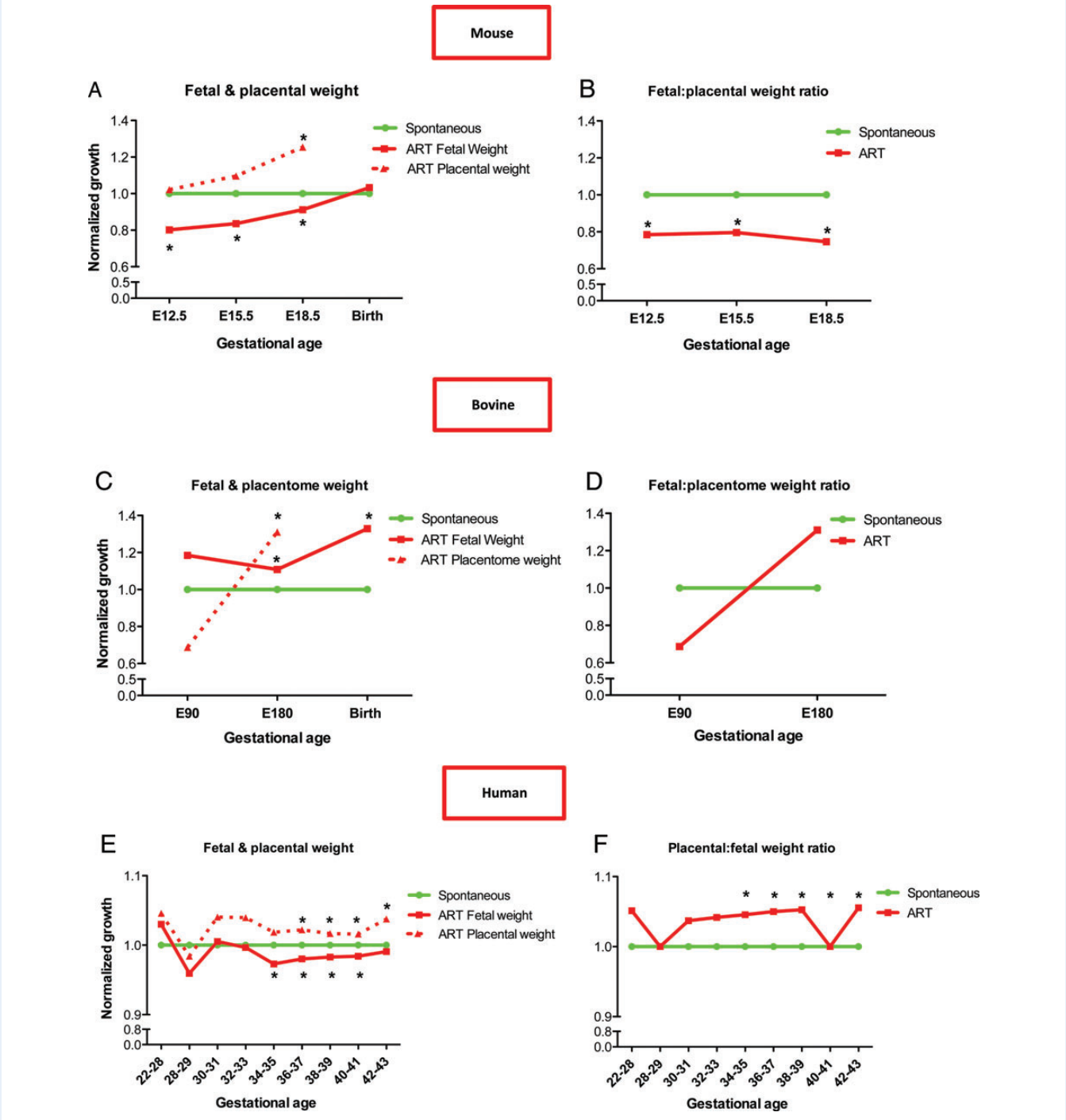

Figure 3 Intrauterine growth restriction, placentomegaly and accelerated fetal growth in mammalian ART pregnancy. Mean fetal and placental ART weights relative to spontaneously conceived concepti, with corresponding placental:fetal weight ratios in mouse (**A** and **B**), bovine (**C** and **D**) and human (**E** and **F**) pregnancies. Adapted from mouse: Delle Piane *et al.* (2010) and Bloise *et al.* (2012); bovine: Bertolini *et al.* (2004); human: Haavaldsen *et al.* (2012). *$P < 0.05$. E, embryonic day. Gestational age is days for mouse (A,B) and bovine (C,D) and weeks for humans (E,F).

0.9%, respectively. No differences were found in F:P ratio between IVF and ICSI concepti. An important feature of this study is that the data for placental and fetal weights and ratios were averaged from individual gestational ages categorized as 22–27 weeks, 28–29, 30–31, 32–33, 34–35, 36–37, 38–39, 40–41, and 42–43 weeks of age at birth. Analysis by each week of pregnancy demonstrated that ART birthweights are lower for babies born at 34–41 weeks of gestation. However, birthweights in ART-conceived pregnancies were no longer different in fetuses born after 42 weeks, which could possibly reflect intrauterine catch-up growth in post-term pregnancies (Fig. 3).

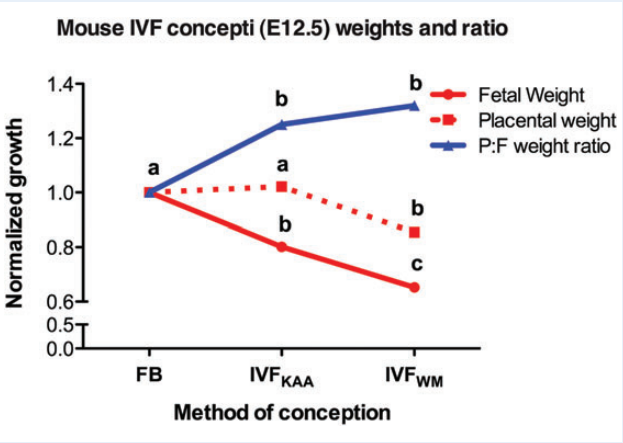

Figure 4 Increased IVF stress exacerbates fetoplacental phenotype. Mouse E12.5 mean fetal weight (red line), placental weight (red dotted line), and corresponding placental:fetal (P:F) weight ratio (blue line) of concepti conceived by IVF and cultured either under optimized conditions (KSOM medium with amino acids under 5% O_2, IVF$_{KAA}$) or suboptimal culture conditions (Whitten's medium under 20% O_2, IVF$_{WM}$), normalized to *in vivo*-derived blastocysts transferred to surrogate damns (flushed blastocyst control, FB). Different superscripts indicate statistical significance between spontaneously conceived pregnancies and IVF concepti produced in different media as shown in the original reports ($P < 0.05$). Adapted from Delle Piane *et al.* (2010).

In summary, there is a species-specific effect of ART on intrauterine growth rates. Birthweight in mouse IVF concepti is variably smaller or larger than controls in a culture condition-, strain- and/or stage at transfer-specific manner, whereas embryo manipulation in bovine models is associated with higher birthweights (Bertolini *et al.*, 2002, 2004; Farin *et al.*, 2006). In all of these species, fetal catch-up growth is apparent by mid-pregnancy and continues until birth, which may be related to adaptations in placental phenotype, including placentomegaly. In humans, IVF gestations are marked by lower birthweights and increased placental size, and may too show evidence of accelerated intrauterine growth in later stages of gestation (Walker *et al.*, 2000; Rinaudo and Lamb, 2008; Haavaldsen *et al.*, 2012). Low birthweight is an established marker of intrauterine stress and has been linked to coronary heart disease, hypertension and hyperlipidemia, even if within the spectrum of 'normal' birthweight (de Boo and Harding, 2006; Meas, 2010). Moreover, a relationship between low birthweight and altered glucose and insulin metabolism in adulthood has been described (de Boo and Harding, 2006; Rinaudo and Wang, 2012). Both impaired fetal growth and accelerated perinatal growth velocity are appreciable signs of fetal stress, and the DOHaD hypothesis emphasizes these features as susceptibility markers of adult disease (Meas, 2010). In fact, many animal studies have demonstrated a strong association between prenatal growth deficit, compensatory growth and increased susceptibility to cardiometabolic defects in adulthood (Armitage *et al.*, 2004). It is difficult to uncouple whether the risk for metabolic syndrome in adult life originates in embryo/fetal adaptations to suboptimal environments during critical periods of development, or instead in the ensuing accelerated growth, or both. Jimenez-Chillaron and colleagues reported that prevention of post-natal catch-up growth in low birthweight mice reversed adult phenotypes of glucose intolerance and diabetes (Jimenez-Chillaron *et al.*, 2006), suggesting that growth kinetics in early life are particularly relevant to adult metabolic physiology. The reasons for altered fetal and placental growth in ART pregnancies are unclear and could be secondary to the underlying infertility affecting people using ART procedures (Romundstad *et al.*, 2008). However, animal data, in which subfertility is not a variable, point to a specific effect of the technology used (for a review see Feuer and Rinaudo, 2012). The data additionally demonstrate that birthweight—a supposed marker of fetal growth trajectory—may not be a reliable indicator of fetal stress (Feuer *et al.*, 2013).

Morphology and Growth of ART Placentae

The evidence that preimplantation embryo manipulation *in vitro* alters normal F:P weight ratio is indicative of fetal exposure to intrauterine stress. To ensure sufficient support of fetal growth and prevention of fetal demise, adaptive placentomegaly is a reasonable mechanism underlying the intrauterine catch-up growth observed in mouse ART concepti. Histological analyses of mouse IVF placentae at E12.5 (Delle Piane *et al.*, 2010) and E18.5 (Raunig *et al.*, 2011a; Bloise *et al.*, 2012) did not reveal any differences in gross morphology. There were no changes in labyrinth or spongiotrophoblast cell number, in spite of the marked placentomegaly present by E18.5 (Raunig *et al.*, 2011a; Bloise *et al.*, 2012). At both time points, the two layers in IVF placentae displayed significantly more Ki67-positive cells, marking an increase in cellular proliferation. This suggests that placental overgrowth observed in term ART pregnancies is secondary to increased proliferation detectable at mid- to late gestation.

It is also possible that hyperproliferation of labyrinth and spongiotrophoblast cells (Delle Piane *et al.*, 2010; Bloise *et al.*, 2012) is a compensatory response to inflammation and oxidative stress. Mouse ICSI and IVF placentae are marked by increased interleukin (IL)-6 levels, indicative of placental inflammation; complementarily, ICSI placentae exhibit impaired activities of the antioxidants superoxide dismutase, thioredoxin reductase, xanthine oxidase, catalase, glutathione-S-transferase, glutathione peroxidase and glutathione reductase (Raunig *et al.*, 2011b). Abnormal antioxidant defense networks were also observed in fetal livers. Because local inflammation and oxidative stress can trigger cell death (apoptosis), changes in oxidative stress and mechanisms of reactive oxygen species disposal may contribute to the histological phenotypes observed in ART concepti. It is therefore tempting to speculate that increased proliferation is a compensatory response to oxidative stress-induced apoptosis in ART placentae, in order to prevent fetal demise. In fact, the same report showed increased placental apoptosis after IVF and ICSI (Raunig *et al.*, 2011b), although a separate study did not observe any changes in apoptosis levels following IVF and embryo culture (Bloise *et al.*, 2012). These divergent results may be related to the sensitivity of the techniques or conditions of embryo culture and transfer (discussed below).

Bovine ART is also characterized by placental overgrowth. *In vitro*-derived concepti were supported by placentomes with increased diameter and decreased thickness compared with controls (Bertolini *et al.*, 2002). This phenotype was present in early pregnancy (E72 to E93) and maintained to term, at which point the longer, thinner placentomes additionally displayed larger surface areas due to the presence of giant cotyledon structures, which were correlated with the LOS. In another report, no alterations in placental morphometry or stereological

parameters were described for third trimester IVF concepti (E229) relative to artificially inseminated (AI) animals (Constant *et al.*, 2006). Conversely, SCNT placentomes displayed increased volume density of fetal connective tissue and decreased volume density of the maternal epithelium, demonstrating that the growth of the maternal and fetal components of the placentomes were not similar (Constant *et al.*, 2006).

Sufficient invasion into the uterine epithelium and appropriate vascularity are essential components of placental function, as the maternal:fetal interface is the basis for all communication and fetal support. It follows that without proper vascularization, placental growth and activity may be impaired, leading to a compromised fetal environment and the inability to meet the evolving demands of a growing fetus. Microarray analysis of E50 IVF- and SCNT-derived bovine placentae compared with artificial insemination-derived control placentae identified 58 genes commonly misexpressed in the two experimental groups. These genes were enriched for involvement in organogenesis, extracellular structure and matrix organization, as well as the development, regulation, and maintenance of blood vessels and vasculature (Salilew-Wondim *et al.*, 2013). Another particularly relevant study identified changes in placental expression of vascular endothelial growth factor (VEGF)-A and basic fibroblast growth factor—two major angiogenic factors controlling placental vascularization—in SCNT bovine placentae at term (Campos *et al.*, 2010). Other reported alterations in blood vessel development included decreases in both blood vessel density and levels of *Vegf* mRNA in cotyledonary tissue from IVF bovine concepti (Miles *et al.*, 2005), an effect occurring in a culture medium-specific manner. Desensitized VEGF signaling could underline the impaired blood vessel development, since it has been demonstrated that placentomes from cloned animals are less responsive to VEGF treatments (Sousa *et al.*, 2012). Further, impaired cotyledonary vasculogenesis and angiogenesis during earlier stages of pregnancy could have detrimental effects on subsequent placental development and maturation (Meegdes *et al.*, 1988). For example, elevated expression of *Hif-1*α (hypoxia-inducible factor) in SCNT-derived maternal and chorioallantoic tissues reflects generalized hypoxic conditions, suggesting an insufficient maternal blood supply (Hoffert-Goeres *et al.*, 2007).

Increased placental size and thickness have also been described in human ART pregnancies, independent of ART procedure type, infertility factor, pregnancy complications, perinatal outcome, or fetal weight (Daniel *et al.*, 1999). Other histopathological discrepancies include a significantly higher incidence of villous edema and micro calcifications (Lalosević *et al.*, 2003), increased thickness and a higher prevalence of hematomas (Joy *et al.*, 2012), which lead to greater risk of perinatal complications (Elchalal *et al.*, 2000). There is a greater frequency of abnormal cord insertion (Daniel *et al.*, 1999), which has previously been related to ART-associated IUGR (odds ratio: 3.69) (Cai *et al.*, 2006). A comparative study of placental blood barrier, fetal capillaries, villous stroma, cytotrophoblast and syncytiotrophoblast substructure by transmission-electron microscopy in eight ART and fifteen spontaneously conceived term placentae identified several ultrastructural changes (Zhang *et al.*, 2011). ART placentae showed degenerative alterations to terminal villi (predominantly in the syncytiotrophoblast lineage), including thicker placental barrier, fewer apical microvilli and a greater number of multiple vacuoles. These abnormalities at the maternal:fetal interface could reflect impaired establishment of the placental blood barrier, a hypothesis further supported by proteomic analyses identifying changes in membrane trafficking, cytoskeletal integrity, metabolism, the stress response, and nucleic acid processing in IVF and ICSI placentae (Zhang *et al.*, 2008). Subsequent microarray analysis further distinguished ART samples by abnormal transmembrane transport, metabolism, oxidative stress, immune response and cell differentiation gene expression (Zhang *et al.*, 2010).

In summary, it is clear that ART procedures impact morphology and growth of ART placentae in different species. The extent to which these changes are present varies according to the species investigated and methodologies applied. Whether these differences play a causal role in the fetal growth alterations or are secondary to other ART-induced modifications remains to be elucidated. Table II summarizes the placental phenotypes described in ART concepti.

Changes to Placental Nutrient Transport

Critical to proper placental function in support of fetal growth is nutrient transport capacity. Accordingly, altered conceptus growth velocity and weight in ART pregnancies may be linked to changes in placental transport efficiency, leading to impaired support of fetal growth (Table III). Insufficient nourishment would precipitate IUGR of the fetus, as well as compensatory placentomegaly. Placental function and nutrient transport were recently explored in a study monitoring fetal accumulation of glucose and neutral amino acids in IVF mouse embryos. Maternal intrajugular injection of ^{14}C-methyl aminoisobutyric acid (^{14}C-MeAIB), a *sytem A* amino acid transporter substrate (SNAT: see below), revealed a 58.1% decrease in placental transport for the IVF group compared with controls ($P < 0.05$), with a corresponding 36% decrease in fetal accumulation of MeAIB ($P < 0.05$). (Bloise *et al.*, 2012). No changes were observed in placental glucose transport following maternal intrajugular injection of ^{14}C-methyl-D-glucose between IVF and control mice (24.8% lower radiolabeled glucose in IVF placentae and 3% lower in IVF fetuses, not significant).

Changes to SNATs have been broadly correlated with pathologically growth-restricted fetuses (Mahendran *et al.*, 1993; Glazier *et al.*, 1997; Harrington *et al.*, 1999; Jansson *et al.*, 2002). Impaired SNAT activity has been observed in placentae from IUGR-complicated pregnancies (Jansson *et al.*, 2002). The expression and activity of SNAT transporters are involved in modifying net placental nutrient transport capacity in coordination with placental size (Coan *et al.*, 2008). SNATs are regulated by glucocorticoids (Audette *et al.*, 2010, 2011), hypoxic conditions (Nelson *et al.*, 2003) and nutrition (Jones *et al.*, 2009), indicating their ability to transduce a myriad of environmental signals and facilitate adaptations in placental nutrient transport ability appropriately. In mice generated by IVF, *Snat2* and *Snat4* mRNA levels were down-regulated in whole placentae at embryonic day E18.5, which may contribute to the impaired transport of neutral amino acids observed in IVF concepti (Bloise *et al.*, 2012).

Amino acids not only provide substrates for protein synthesis, but also serve as metabolic energy sources during fetal development. In fact, it is estimated that upwards of 20–40% of the total energy supplied to the fetal/placental unit is derived from amino acids (Bauer *et al.*, 1998; Cleal and Lewis, 2008). Moreover, the quantity and composition of amino acids supplied to the fetus play an important role in determining fetal growth, as fetuses have specific and evolving metabolic requirements for certain amino acids across gestation (Cleal and Lewis,

Table II Placental phenotype and molecular alterations described in ART concepti.

Species	ART	Placental phenotype	Molecular alterations	References
Mouse	IVF	⇑ proliferation at labyrinth and junctional zones; ⇑ number of apoptotic cells	Abnormal antioxidant defense network; Alterations in placental transcriptome; Hypomethylation at the *H19* locus	Doherty *et al.* (2000), Mann *et al.* (2004), Delle-Pianne *et al* (2010), Faunque *et al.* (2010), Raunig *et al.* (2011a, b) and Bloise *et al.* (2012)
	ICSI	⇑ number of apoptotic cells	Abnormal antioxidant defense network	
Bovine	IVF	Longer and thinner placentomes during early pregnancy (E72 to E93); Larger cotyledonary surface and giant cotyledon structures at term; Heavier placentomes at E180 and ⇓ blood vessels density	⇓ levels of cotyledonary *Vegf* mRNA	Bertolini *et al.* (2002), Bertolini *et al.* (2004) and Miles *et al.* (2005)
	SCNT	Reduced number of placentomes; edematous and/or hemorrhagic changes; ⇑ volume density of fetal connective tissues; ⇓ volume density of maternal epithelium	Alterations in placental transcriptome; ⇑ caruncular and chorio-allantoic *Hif1α* mRNA expression; Desensitization of VEGF signaling in placentomes	Chavatte-Palmer *et al.* (2002), Hoffert-Goeres *et al.* (2007), Chavatte-Palmer *et al.* (2012), Sousa *et al.* (2012) and Salilew-Wondim *et al.* (2013)
Human	IVF	⇑ placental thickness; ⇑ incidence of hematomas and villous edema; ⇑ incidence of micro calcifications	Hypomethylation at the *H19* locus	Lalosević *et al.* (2003), Joy *et al.* (2012) and Nelissen *et al.* (2013)

VEGF, vascular endothelial growth factor; Hif-1α, hypoxia-inducible factor 1-alpha.

Table III Summary of placental nutrient transport in ART concepti.

Species	ART	Fetal:placental weight ratio	Placental nutrient transport	Transporters expression	References
Mouse	IVF	⇓ as of E12.5	⇓ neutral amino acid Transport at E18.5; No change in glucose transport	⇓*Glut1*, *Glut3*/GLUT3, *Snat2* and *Snat3* mRNA	Delle-Pianne *et al.* (2010) and Bloise *et al.* (2012)
Bovine	IVF	⇓ at gestational days E70 and E180.	⇓ fetal plasma glucose concentrations at E90; ⇑ plasma fructose concentrations soon after birth	⇔ *Glut1* and *Glut3* mRNA expression	Bertolini *et al.* (2002) and Bertolini *et al.* (2004)
	SCNT	⇓ after E220	⇔ fetal glucose concentrations; ⇓ allantoic fluid glucose and fructose levels	⇑ *Glut1* and *Glut3* mRNA expression	Hirayama *et al.* (2011) and Chavatte-Palmer *et al.* (2012).
Human	IVF/ ICSI	⇓ as of 34th week of gestation	–	⇑ *SNAT1* mRNA expression	Haavaldsen *et al.* (2012) and Zhang *et al.* (2010)

SNAT, *sytem A* amino acid transporter substrate.

2008). The reduced availability of amino acids in ART placentae and subsequent decrease in amino acid delivery to the fetus could lead to reprogramming of fetal metabolism, including adaptations toward the utilization of different metabolic pathways to better support fetal growth. However, the molecular mechanisms underlying these metabolic changes and reduced amino acid transport are unknown and could be diverse (Seckl and Holmes, 2007).

Changes in steroid efflux have also been described in ART pregnancies. Collier and co-workers showed that IVF- and ICSI-derived placentae displayed different activities of the steroid metabolizing enzymes UDP-glucuronosyltransferase and sulfotransferase, as well as the steroid regenerating enzymes β-glucuronidase and aryl sulfatase. These changes were correlated with higher levels of the steroid metabolites androstane-3α-17β-diol glucuronide and dehydroepiandrosterone sulfate in fetal blood from IVF and ICSI pregnancies (Collier *et al.*, 2009). In another study, the same group reported diminished cholesterol levels in IVF and ICSI placentae (Raunig *et al.*, 2011a). Interestingly, there were no differences in cholesterol concentration in the fetal compartment. Because cholesterol is vital to proper embryo patterning and development (Raunig *et al.*, 2011a), it is possible that placental/fetal adaptations favor cholesterol accumulation in the fetus at the forfeit of cholesterol deposition in the placenta; alternatively, the placental changes may be compensated for through increased fetal synthesis of cholesterol de novo.

Because fetal glucose production is minimal, fetal glucose supply depends almost exclusively on the placenta. Investigation in bovine models has revealed evidence of abnormal intrauterine carbohydrate metabolism following ART. At E90, IVF concepti were growth-restricted with significantly reduced fetal glucose, but by E180 IVF fetuses outweighed controls (with parallel increases in placentome size) and glucose levels were restored (Bertolini *et al.*, 2004). Interestingly, there

were no changes in IVF placentome expression of the glucose transporters *Glut1* or *Glut3*. Consistent with the larger placental phenotype in mice, this suggests that changes in placental weight might contribute to the restored glucose concentrations. Comparatively, SCNT-derived fetuses did not display different plasmatic glucose levels at term relative to *in vivo*-derived controls, but did exhibit up-regulated caruncular *Glut1* and *Glut3* expression (Hirayama *et al.*, 2011), indicating that the relationship between mRNA expression and metabolite concentration in the bovine placenta is complex. Further, fructose concentrations were increased in E180 IVF fetal plasma. It is therefore possible that in conjunction with increased placentome growth, alternative carbohydrates may be exploited to combat early pregnancy fetal IUGR, leading to restored fetal glucose levels, accelerated fetal growth and larger offspring outcome. (Bertolini *et al.*, 2004).

Unfortunately, very little is known about placental nutrient transport in human ART pregnancies. Microarray analysis of IVF human placentae at term identified an over two-fold increase in mRNA expression of the SNAT 1 neutral amino acid transporter (Solute carrier family 38, member 1 – *SLC38A1*) compared with spontaneously conceived pregnancies (Zhang *et al.*, 2010). Increased placental *SLC38A1* amino acid transporter expression may be an important adjustment toward ensuring that ART concepti meet their nutrient requirements across pregnancy, although this remains to be determined. Further investigation is warranted in order to clarify whether placental nutrient transport is misregulated in human ART-derived pregnancies.

Preimplantation Determinants of Intrauterine Growth: How ART Procedures May Affect Development

Multiple factors are involved in establishing a dialogue between the developing conceptus and maternal environment in support of a healthy pregnancy, including gamete maturation, the process of fertilization, preimplantation development, blastocyst apposition and invasion into the uterine epithelium. ART introduces several variables that may affect these factors and lead to the disrupted growth trajectories described above. Moreover, the tubal and uterine environments, which accommodate embryos through cleavage and blastocyst development, respectively, are markedly different from one another with regard to nutrient availability, pH and oxygen tension; this dynamic metabolic environment is lost with culture *in vitro* (Fig. 2) and can negatively impact blastocyst viability (Feuer and Rinaudo, 2012). This section will describe the effect of ART on variables with known importance to fetomaternal harmony, with specific attention to blastocyst growth and proliferative potential, gamete and embryo metabolism, genomic imprinting, and uterine receptivity (Fig. 5).

Blastocyst cell number and developmental competence

The developmental potential of the blastocyst predicts post-implantation capacity, and ART has a well-described effect on blastocyst formation, viability and cell number. Embryo culture generally introduces an 18–24 lag in blastocyst development, and various components of embryo culture become key determinants of embryonic developmental competence and ART success rates (Rinaudo and Schultz, 2004; Rinaudo *et al.*, 2006). As culture conditions better recapitulate the endogenous environment, blastocyst rates increase, predisposing higher pregnancy and live birth rates. For example, the oviduct and uterine environments are stratified by pH approximately >7.5 and <7.1, respectively, with slight variations across different mammalian species (Feuer and Rinaudo, 2012). Lowering the culture medium pH from 7.3 after 48 h of culture to 7.15 improved blastocyst outcome over embryos maintained at one pH (Hentemann *et al.*, 2011). What is more, even small variations in culture conditions exert differential effects on development, as exemplified by a comprehensive analysis of the individual effects of 13 different human IVF culture protocols on mouse blastocyst development and proliferation by Schwarzer and colleagues (Schwarzer *et al.*, 2012). They observed condition-specific consequences on blastocyst and fetal developmental rates, cell numbers, gene expression signatures, and litter sizes, indicating that particular elements of culture have measured and defined outcomes for cell proliferation, lineage specification and embryo viability.

It is well appreciated that blastocyst proliferation and cell lineage pool size impact fetal development and viability, with decreased cell number correlating with impaired fetal competence. Mouse blastocysts comprising fewer cells have reduced implantation rates and litter sizes, and yield an increased number of abortive sites (Delle Piane *et al.*, 2010; Bloise *et al.*, 2012). Moreover, reduced blastocyst cell number signifies fewer progenitors contributing to the fetal and extraembryonic derivative lineages (ICM and trophectoderm [TE] cells, respectively), which may lead to several deleterious consequences for fetal growth. Studies in mouse embryos have shown that artificial reduction of preimplantation embryo cell number can significantly delay gastrulation, morphogenesis and somite segmentation (Power and Tam, 1993). This rescheduling of early post-implantation events may precede the accelerated compensatory intrauterine growth observed in later stages of ART pregnancies. Decreases in blastocyst cell number after *in vitro* culture of bovine embryos is additionally correlated with impaired developmental potential, which may precede the LOS phenotype (Van Soom et al., 1997). A recent study in humans demonstrated that TE morphologic grade (based in part off cell number) is a stronger predictor of successful ART outcome than ICM grade; this may be related to optimal ploidy or improved blastocyst-endometrium communication (Hill *et al.*, 2013).

Because ICM and TE cells have extraordinarily different fates, behaviors, morphologies and functions, many ART-induced changes may be masked by analyses of whole embryos. Microarray profiling of ICM and TE transcriptional signatures have revealed significant differential expression between the two lineages for pathways of differentiation and pluripotency, cell signaling, molecular transport, transcriptional regulation, and metabolism in mice (Giritharan *et al.*, 2012), bovines (Ozawa *et al.*, 2012), and humans (Adjaye *et al.*, 2005). Although very few studies have investigated the transcriptional changes specific to ICM or TE cells following ART, embryos produced by IVF (mice) and SCNT (bovine) both show drastically reduced differential expression between ICM and TE lineages, specifically for genes involved in development and pluripotency (Fujii *et al.*, 2010; Giritharan *et al.*, 2012). Detailed analysis of the individual cell fates in mouse embryos revealed an impact of IVF on ICM cell pluripotency, phospholipid metabolism, liptoxicity and urea cycle gene expression signatures (Giritharan *et al.*, 2012). Comparably, IVF largely down-regulated TE expression of genes functioning in solute transport and placentation. Loss of gene signatures distinguishing ICM versus TE segregation as well as differentiation and placentation

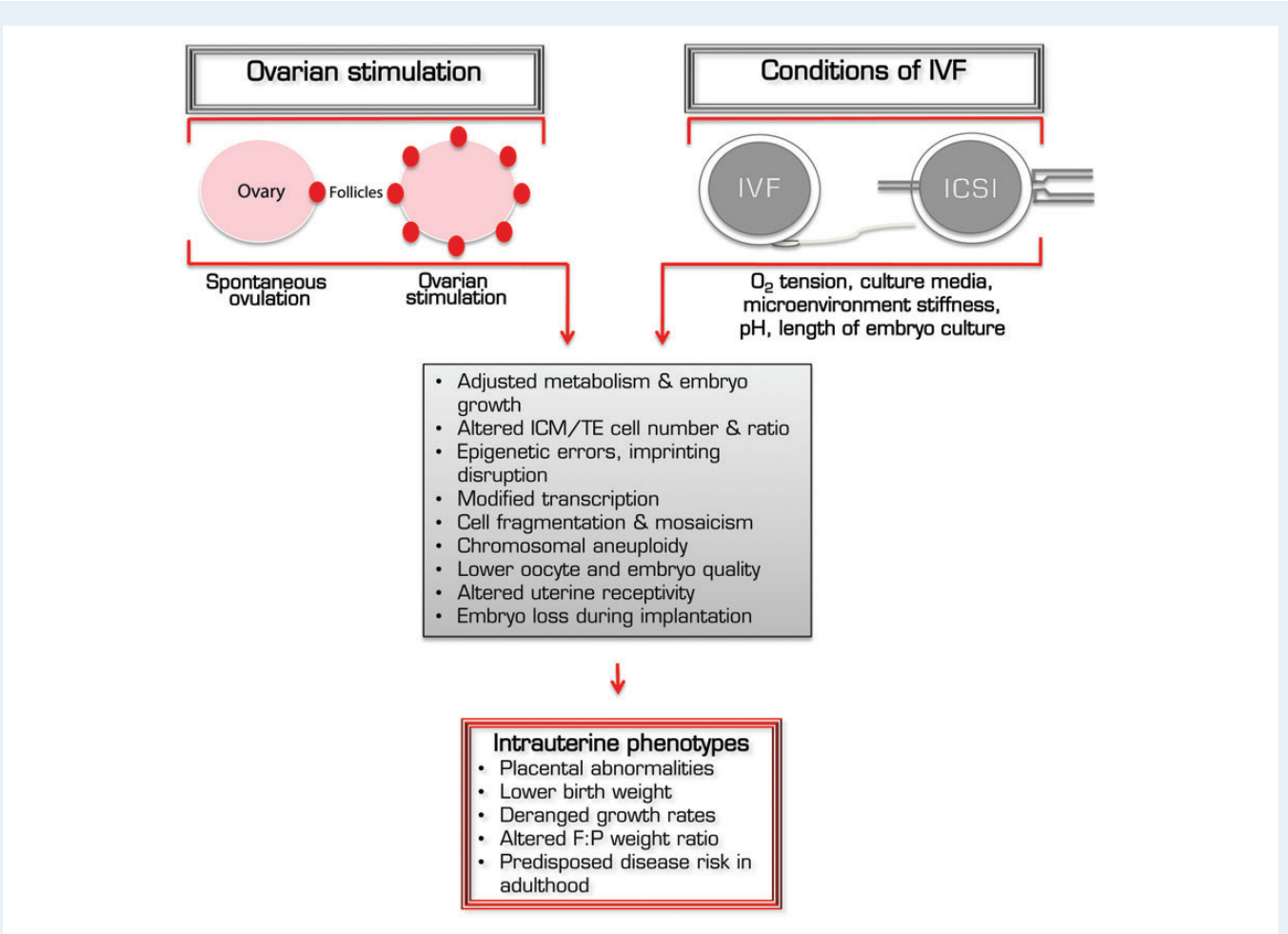

Figure 5 Mechanisms by which ART procedures affect acute and long-term growth. Both ovarian stimulation with exogenous gonadotrophins as well as assorted components of IVF and embryo culture conditions, including oxygen tension, culture medium composition, culture substrate rigidity, pH and the duration of embryo culture, can affect oocyte and embryo developmental competence. This can lead to epigenetic errors, cell mosaicism and ploidy defects, while also inducing adjustments to embryo metabolism, transcription and proliferation. Cumulatively, these acute outcomes can influence intrauterine growth kinetics, leading to the fetal and placental phenotypes observed in ART offspring, and possibly subsequent increased risk of metabolic disease in adulthood. ICM, inner cell mass; TE, trophectoderm.

processes would contribute to the altered lineage ratios and fetoplacental growth abnormalities observed *in utero*. In fact, is has been suggested that alterations to TE gene expression could be in part responsible for altered placental size found in human ICSI-derived pregnancies (Haavaldsen *et al.*, 2012).

The sensitivities of ICM:TE number and overall blastocyst viability to the individual effects of specific embryo culture components, including culture medium composition, oxygen tension and microenvironment stiffness/rigidity, have been the subject of numerous studies. Outstandingly, more severe culture conditions yield more deviant phenotypes (Rinaudo *et al.*, 2006; Kolahi *et al.*, 2012; Schwarzer *et al.*, 2012).

Oxygen tension

In the mammalian reproductive tract, oxygen concentrations change from ~8% at the time of fertilization in the oviduct, to ~1–2% in the uterus (Bavister, 2004). However, this gradient is abolished with embryo culture, and several reports have argued the harmful effects of higher oxygen concentrations on preimplantation development.

Culture of mouse zygotes in 20% (atmospheric) compared with 5% oxygen levels significantly reduced the number of embryos reaching the blastocyst stage, as well as number of ICM cells and total cell number (Rinaudo *et al.*, 2006). Similarly, percentage oxygen during *in vitro* maturation of oocytes can affect blastocyst rate, TE cell number and apoptosis levels, as well as fetal and placental weights (Banwell *et al.*, 2007). Further, oxygen tension has a pronounced effect on gene expression in blastocysts. Culture of mouse zygotes to blastocysts in optimized media conditions (KSOM supplemented with amino acids) under 5 versus 20% oxygen tensions induced significant changes to 29 and 354 genes, respectively, compared with *in vivo*-produced embryos. The same experiment using a suboptimal medium, Whitten's medium, affected the expression of 114 genes (5% oxygen) and 1159 genes (20% oxygen), and was additionally associated with decreased blastocyst rates and cell numbers (Rinaudo and Schultz, 2004; Rinaudo *et al.*, 2006). This indicates a synergism between culture medium and oxygen concentration, such that the detrimental effects of suboptimal media are severely compounded by higher oxygen tensions. Moreover, high

oxygen during culture was linked with increased abortion rates, decreased fetal and placental weights, and higher F:P ratio (Delle Piane *et al.*, 2010).

The stressful effects of high oxygen have also been shown in bovines. Lowering the oxygen concentration from ambient to 5% correlated with increased blastocyst rates and expression of genes involved in glucose transport, mitochondrial function and integrity, and antioxidant defense (Balasubramanian *et al.*, 2007). In contrast, atmospheric oxygen induced higher transcript levels of oxidative stress genes. This indicates that high oxygen produces a stress response in the embryo, which substantiates the fact that lower oxygen tensions mimic a more physiological preimplantation environment.

A meta-analysis of seven randomized human ART studies associated physiologic oxygen concentrations with greater percentages of blastocyst formation and significantly increased implantation rates ($P = 0.006$) (Gomes Sobrinho *et al.*, 2011). Other studies not included in this analysis have corroborated higher blastocyst development, implantation, and pregnancy rates for human embryos cultured in low oxygen (Catt and Henmam, 2000; Kea *et al.*, 2007; Meintjes *et al.*, 2009; Waldenström *et al.*, 2009; Kovacic *et al.*, 2010). A separate meta-analysis similarly reported increases in clinical pregnancy, ongoing pregnancy, and live birth rates for 5% compared with 20% culture conditions, with no effect on multiple pregnancy, miscarriage, or congenital anomaly risk (Bontekoe *et al.*, 2010). To date, an effect of oxygen tension on birthweight has not been systematically reviewed. Interestingly, embryos transferred on Day 2 or 3 of development display no changes in fertilization rate ($P = 0.54$), implantation rate ($P = 0.63$), or ongoing pregnancy rate ($P = 0.19$) (discussed below) (Gomes Sobrinho *et al.*, 2011).

Overall, the data described in this section indicate that oxygen tension is a major factor regulating embryo growth in different species. Although some studies comparing the impact of low versus high oxygen concentrations on pregnancy outcome remain controversial, we wish to advise in favor of culturing embryos in a low-oxygen environment when performing ART.

Length of embryo culture

The efficacy and safety of cleavage (Day 3) versus blastocyst (Day 5) stage transfer of human embryos is one of the most discussed controversies in reproductive medicine today. Evidence in the literature demonstrates that clinical pregnancy and live birth rates from Day 5 transfer are superior (Papanikolaou *et al.*, 2006, 2008; Blake *et al.*, 2007), but blastocyst transfer is additionally linked with increased prenatal complications, preterm birth, low Apgar score, respiratory diagnoses, and congenital malformations (Milki *et al.*, 2003; Källén *et al.*, 2010; Maheshwari *et al.*, 2013; Dar *et al.*, 2014). Additionally, duration of embryo culture is a highly significant independent factor determining birthweight in humans: weights are markedly higher following blastocyst transfer (Zhu *et al.*, 2014), and the percentage of babies born large for gestational age nearly doubles for Day 5 compared with Day 2/3 transfer (Makinen *et al.*, 2013).

One explanation for the length of embryo culture effects is the sensitivity of the blastocyst after cell segregation into the ICM and TE lineages. There is a well-defined role for oxygen in trophoblast proliferation and differentiation (reviewed in Red-Horse *et al.*, 2004). The earliest stages of placentation favor hypoxic conditions, which support cytotrophoblast expansion; as these cells invade the uterine epithelium, exposure to higher oxygen levels from the maternal blood supply favors their differentiation (Genbacev *et al.*, 1997). Further, ablation of different oxygen-sensing or -responsive proteins—including *von Hippel Lindau* or HIF-1β—compromises placental cell fate determination, leading to fetal demise secondary to placental defects (Gnarra *et al.*, 1997; Adelman *et al.*, 2000). A study using trophoblast stem cells derived from TE showed that HIF is specifically involved in modulating epigenetic changes that govern trophoblast differentiation (Adelman *et al.*, 2000). It is possible that culturing embryos in ambient oxygen predisposes to impairments to placentation and subsequent intrauterine growth, whereas the uterine environment abrogates the deleterious effects of high oxygen concentrations in embryos tranferred on Day 2/3. This is further evidenced by an effect of high oxygen exposure (compared with hypoxic conditions, 2% oxygen) exclusively during the morula-blastocyst transition on F:P weight ratio (Feil *et al.*, 2006). It would be interesting to assess viability, cell number, and developmental stress in IVF blastocysts previously transferred as cleavage embryos, to determine if the final stages of preimplantation development *in vivo* are sufficient to rescue ART-induced changes present earlier.

Rigitidy of the culture substrate

Another important feature of the preimplantation microenvironment is surface stiffness. A conventional polystyrene petri dish (PD) has a measured elasticity of 1 GPa whereas the uterine epithelium has a ~1kPa stiffness—a difference in rigidity of over 10^6. Culture of mouse embryos either on polydimethyl-siloxane-coated PDs or fabricated 3D type I collagen gels (both with elasticities of 1kPa) resulted in a significantly greater frequency of development to the 2-cell, blastocyst, and hatching blastocyst stages compared with control PD embryos (Kolahi *et al.*, 2012). Further, 1kPa conditions were associated with increased blastocyst cell number, particularly within the TE lineage. This further indicates that blastocyst developmental potential improves as culture conditions better mimic the female reproductive tract.

Gamete and preimplantation embryo metabolism

The evolution of zygote to blastocyst occurs over a dynamic range of nutrients, oxygen, and growth signals distinguishing the oviduct from uterus. Therefore, the progression between the two microenvironments requires a metabolic plasticity that is achieved through flexible management of glycolytic activity, oxidative phosphorylation, and membrane transport (Gardner and Leese, 1988; Leese, 2012). For example, as the zygote transitions to blastocyst, its metabolic activity preferentially shifts from pyruvate to glucose oxidation, with parallel increases in glucose availability upon entry into the uterus. However, the absence of glucose in culture prevents the characteristic decline of pyruvate uptake after compaction (Brinster, 1965). This adaptability confers acute survival advantages, yet in more stressful or artifical environments it may not be optimal long term.

In the mouse, TE cells generate ~80% of total blastocyst ATP production and are responsible for 90% of the embryo's amino acid turnover, primarily to fuel the sodium transporter enzymes that facilitate cavitation (Houghton, 2006). It follows that reduced TE cell number would have a profound impact on embryo integrity. Additionally, impaired proliferation can denote metabolic or oxidative stress, prompting the redistribution of cellular resources away from proliferative activities and alternatively toward repair pathways (i.e. the quiet embryo

hypothesis) (Leese, 2012). This is evidenced by the observation that embryo culture is associated with an increase in mitochondrial activity, amino acid turnover, and tandem decreased expression of genes involved in shunting glycolytic intermediates toward biosynthetic processes versus the TCA cycle (and ATP syntheis) (Krisher and Prather, 2012; Redel *et al.*, 2012).

In all reports investigating the effects of IVF, ICSI, and embryo culture on blastocyst gene expression, the majority of the transcriptional changes are to genes involved in metabolic processes (Rinaudo and Schultz, 2004; Rinaudo *et al.*, 2006; Giritharan *et al.*, 2007, 2010, 2012; Schwarzer *et al.*, 2012). In particular, studies investigating the effects of culture medium composition on preimplantation development have revealed that growth and cell fate in the early embryo is sensitive to the availability of different metabolic substrates (carbohydrates, amino acids), which can consequentially affect post-implantation development. In fact, the addition of exogenous pyruvate and lactate to the medium during the pronuclear stage (and subsequent manipulation of redox potential) alters birthweights and post-natal growth curves of mouse ART concepti (Banrezes *et al.*, 2011).

Moreover, different forms of exogenous gonadotrophin preparations can impact oocyte and embryo metabolism. Following controlled ovarian stimulation, oocytes progressing to the metaphase II stage exhibited unique amino acid turnover profiles depending on their exposure to recombinant FSH versus hMG (a urinary gonadotrophin) treatments (Hemmings *et al.*, 2013). Because oocyte metabolic state predisposes fertilization and developmental competency (Hemmings *et al.*, 2012), hormone-induced changes to oocyte metabolism could pervasively influence future growth potential. Correspondingly, ovulation induction impairs embryo development to blastocyst and also affects global expression of genes involved in growth and proliferation, including DNA and protein synthesis, transcription, RNA post-transcriptional modification, and other bioenergetic pathways supporting anabolism (Gad *et al.*, 2011).

Imprinted genes and epigenetic changes

As discussed above, IVF and embryo culture introduce significant changes to patterns of gene expression in blastocysts in a condition-specific fashion, and many alternations in transcriptional signatures persist well beyond the ART procedures. For example, culture-specific transcriptional signatures are apparent in placental gene expression (Fauque *et al.*, 2010; Schwarzer *et al.*, 2012). Maintence of ART-induced changes following the preimplantation period suggests the existence of a 'memory' of the ART event that impacts subsequent intrauterine and posnatal growth.

It is widely believed that the mechanism of reprogramming is epigenetic in nature. Epigenetic regulation occurs at the DNA level predominantly through the methylation of cytosine bases residing in CpG dinucleotides, or by post-translational modification of histone proteins. These covalent moieties merge into combinatorial signatures that affect chromatin conformation, DNA accessibility, and gene expression through a variety of mechanisms. Importantly, embryogenesis is marked by significant chromatin remodeling in conjunction with cell differentiation, making the preimplantation stages uniquely vulnerable to environmental perturbances (Fig. 1). It is possible that ART procedures induce stochastic changes to epigenetic and transcriptional regulation that become permanently incorporated into programmes of cell fate (Feuer *et al.*, 2013).

It is likely that any epigenetic changes associated with ART occur in a locus-specific fashion. Immunohistochemical analysis of *in vivo* and IVF mouse embryos did not observe any global changes in the levels or distribution of the histone modifications H4 acetylation (H4ac), H3 lysine 9 trimethylation (H3K9me3), or phosphorylated H3 at serine 10 (H3S10p) across all stages of preimplantation development (zygote to blastocyst) (Huang *et al.*, 2007). However, reduced fetal size in early pregnancy has been correlated to impaired placental expression and activity of the methylation maintenance enzyme DNMT1 in IVP sheep placentae, the dysfunction of which was directly related to fetal growth restriction and miscarriage rates (Ptak *et al.*, 2013). Global versus site-specific methylation levels were not evaluated in the ART- and *in vivo*-derived placentae.

Investigators have focused extensively on the effects of ART on imprinted genes, which are expressed monoallelically in a parent-of-origin-dependent manner. This is particularly relevant for genes controlling growth and development, as it affords more stringent regulation of gene dosage. Sometimes this parental allele-based expression is preferential only in a specific subset of tissues, notably the placenta (Tunster *et al.*, 2013). Evidence has emerged demonstrating that imprinted genes are vulnerable to sustained methylation aberrancies following ART, and several excellent reviews have been written on the topic (Manipalviratn *et al.*, 2009; Marchesi *et al.*, 2012).

H19 is an imprinted gene expressed exclusively from the maternally-inherited chromosome, and plays a major role in both limiting placental growth, as well as controlling fetal growth via coordinated nutrient transfer from mother to fetus (Fowden *et al.*, 2006). Several reports have shown that embryo culture can alter *H19* methylation, leading to its biallelic expression in a subset of mouse embryos generated *in vitro*. (Doherty *et al.*, 2000; Mann *et al.*, 2004). Importantly, these alterations (i.e. hypomethylation of the *H19* paternal allele) persisted after implantation, with placental tissue displaying active expression of the normally silent *H19* allele. This indicates that changes in embryo methylation induced by *in vitro* culture can persist in later stages of intrauterine life. Significant *H19* hypomethylation and corresponding increased *H19* mRNA expression have also been observed in human ART placentae (5 IVF and 30 ICSI), although this was not correlated with changes in IVF neonatal birthweight, birthweight standard deviation score, or gestational age (Nelissen *et al.*, 2013). However, *H19* expression defects have been associated with placentomegaly (Bloise *et al.*, 2012; Keniry *et al.*, 2012). As a result, impairments to imprinting mechanisms (including establishment and maintenance of DNA methylation) could have a direct effect on the unique intrauterine growth velocities observed in IVF concepti. A related hypothesis is that errors in DNA methylation may be responsible for the decreased number of TE cells in IVF preimplantation embryos (Rinaudo and Lamb, 2008).

In addition to embryo culture, the administration of exogenous gonadotrophins for ovarian stimulation can affect DNA methylation status at imprinted genes. A recent evaluation of hormone dose on DNA methylation architecture in mouse blastocysts showed a dose-dependent loss of methylation at the maternally imprinted *Snrpn*, *Peg3,* and *Kcnq1ot1* loci, and gain of methylation at the normally hypomethylated maternal *H19* allele, with higher hormone dosages producing greater methylation aberrancies (Market-Velker *et al.*, 2010). Unexpectedly, gonadotrophin exposure resulted in a loss of *H19* methylation at the paternally imprinted allele, a surprising result because ovulation induction is believed to affect genomic imprinting during oocyte development. This suggests an impact

of ovarian stimulation on the acquisition and/or maintenance of imprints across preimplantation development. In a follow-up study, the same group concluded that ovulation induction does not affect methylation acquisition at the examined loci, but instead impairs maintenance of these imprints during preimplantation development (Denomme *et al.*, 2011).

In humans, ovarian stimulation has been linked to a gain of *H19* methylation and a loss of *PEG1* methylation in oocytes, although these results may be confounded by fertility defects in the superovulated ART patients (Sato *et al.*, 2007). A separate report analyzed *H19* methylation profiles in oocytes at various developmental stages following *in vitro* maturation, and a subset of the metaphase II oocytes examined (5 of 20 total) exhibited aberrant methylation signatures (Borghol *et al.*, 2006). Although the specific effects of these changes remain obscure, it is clear that assorted components of ART can induce epigenetic lesions and may compromise the expression of developmentally relevant genes, placental development, fetal growth and viability.

Uterine receptivity

In order to establish the maternofetal crosstalk crucial for a healthy pregnancy, the uterus must accommodate a microenvironment that is both receptive to implantation and supportive of subsequent fetal growth, and the embryo must reciprocate with pregnancy recognition signaling (Bazer *et al.*, 2011). It is becoming increasingly apparent that many pregnancy complications such as pre-eclampsia or preterm delivery—known risks associated with ART—can be traced to abnormalities in implantation and placentation (reviewed in Norwitz, 2006). For example, blastocyst expression of *Il-6* helps facilitate blastocyst-uterine crosstalk and may be involved in trophoblast differentiation and invasion (Norwitz 2006); IVF-derived mouse embryos exhibit a 5.7-fold reduction in TE cell expression of *Il-6* (Giritharan *et al.*, 2012).

Several studies indicate that controlled ovarian stimulation impairs uterine receptivity (Fauser and Devroey, 2003). Horcajadas and colleagues performed microarray analysis of endometrial biopsies obtained from superovulated and natural cycles to elucidate the impact of exogenous gonadotrophins on the early- to mid-secretory transition (Horcajadas *et al.*, 2008). The pre-receptive and receptive phases were distinguished by two separate patterns of gene expression (218 and 133 genes differentiating the two stages, respectively). Ovulation induction induced a 2-day delay in the transition between the two clusters, demonstrating that ovarian stimulation affects the timing of endometrial maturation with possible consequences for receptivity and implantation (Papanikolaou *et al.*, 2005). Differences in ovarian steroid release can also influence the composition of oviduct fluid. Because variations in nutrient and electrolyte availability impact embryo metabolism and growth, this could impair future fetal developmental competence (Murray *et al.*, 1995).

There is also an effect of gonadotrophin dosage on the length of the implantation window (Simon *et al.*, 2003). The luminal endometrial endothelium is responsible for promoting apposition and attachment of the developing blastocyst exclusively during the implantation period. Blastocyst adhesion to the endometrium is predominantly supported either by the addition of progesterone after prior priming with 17β-estradiol (humans), or alternatively by the presence of 17β-estradiol after appropriate priming with progesterone (rodents). Studies in mice have shown that differential doses of exogenous 17β-estradiol can manipulate the duration of the implantation window: administering low levels extend and maintain the window of receptivity, whereas higher doses can rapidly induce a refractory state (Simon *et al.*, 2003).

This may be related to changes in uterine gene expression. Compared with low levels of exogenous progesterone supplementation, exposure to high concentrations reduced endometrial expression of the progesterone and estrogen receptors in cycling heifers (McNeill *et al.*, 2006). Superovulated animals additionally demonstrated sustainably high progesterone levels through Day 6 following artificial insemination compared with unstimulated animals (Gad *et al.*, 2011). It follows that in addition to influencing receptivity for implantation, gonadotrophin administration may induce other endometrial changes that could subsequently affect placentation or maternal recognition of pregnancy.

Conclusions

In conclusion, there is compelling evidence that ART has significant effects on intrauterine growth trajectory and placental support of fetal growth. This is particularly important because fetal growth restriction followed by accelerated perinatal growth velocity mark both fetal stress and a predisposition for cardiometabolic pathologies in adulthood, as described by the DOHaD hypothesis. It is likely that many components of ART procedures, including embryo culture environment and duration, oxygen tension, and gonadotrophin stimulation, impact fetoplacental development through combined effects on oocyte maturation, cell proliferation and specification, blastocyst viability, and endometrial receptivity for implantation. Together these variables could alter maternal recognition of pregnancy that in turn might lead to abnormal placental and fetal growth kinetics. Because differences in fetal intrauterine growth do exhibit species specificity, this highlights the need for focused research on human ART concepti (whenever ethically possible). Specifically, systematic evaluation of fetal growth and placental physiology across gestation with post-natal follow-up are required in order to determine whether ART in humans produces deleterious changes to intrauterine growth trajectory, placental function and subsequent post-natal health.

Authors' roles

E.B. and P.F.R. conceived and designed the study. E.B., S.K.F. and P.F.R. each contributed to performing the search, analyzing the data and writing the manuscript.

Funding

This work was supported by CNPq/Ciencia sem Fronterias (Conselho Nacional de Desenvolvimento Científico e Tecnológico, Brasilia, Brazil), grant number: 402343/2012-3 to E.B. This work was also supported by NIH 5T32-DK007418-32 to S.K.F. and NICHD RO1-062803-01A1 to P.F.R.

Conflict of interest

We declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research we have reported in this manuscript.

References

Adelman DM, Gertsenstein M, Nagy A, Simon MC, Maltepe E. Placental cell fates are regulated *in vivo* by HIF-mediated hypoxia responses. *Genes Dev* 2000; **14**:3191–3203.

Adjaye J, Huntriss J, Herwig R, BenKahla A, Brink TC, Wierling C, Hultschig C, Groth D, Yaspo ML, Picton HM *et al*. Primary differentiation in the human blastocyst: comparative molecular portraits of inner cell mass and trophectoderm cells. *Stem Cells* 2005;**23**:1514–1525.

Armitage JA, Khan IY, Taylor PD, Nathanielsz PW, Poston L. Developmental programming of the metabolic syndrome by maternal nutritional imbalance: how strong is the evidence from experimental models in mammals? *J Physiol* 2004; **561**:355–377.

Audette MC, Greenwood SL, Sibley CP, Jones CJ, Challis JR, Matthews SG, Jones RL. Dexamethasone stimulates placental system A transport and trophoblast differentiation in term villous explants. *Placenta* 2010;**31**:97–105.

Audette MC, Challis JR, Jones RL, Sibley CP, Matthews SG. Antenatal dexamethasone treatment in midgestation reduces system A-mediated transport in the late-gestation murine placenta. *Endocrinology* 2011;**152**:3561–3570.

Aytoz A, Van den Abbeel E, Bonduelle M, Camus M, Joris H, Van Steirteghem A, Devroey P. Obstetric outcome of pregnancies after the transfer of cryopreserved and fresh embryos obtained by conventional in-vitro fertilization and intracytoplasmic sperm injection. *Hum Reprod* 1999;**14**:2619–2624.

Balasubramanian S, Son WJ, Kumar BM, Ock SA, Yoo JG, Im GS, Choe SY, Rho GJ. Expression pattern of oxygen and stress-responsive gene transcripts at various developmental stages of *in vitro* and *in vivo* preimplantation bovine embryos. *Theriogenology* 2007;**68**:265–275.

Banrezes B, Sainte-Beuve T, Canon E, Schultz RM, Cancela J, Ozil JP. Adult body weight is programmed by a redox-regulated and energy-dependent process during the pronuclear stage in mouse. *PLoS One* 2011;**6**:e29388.

Banwell KM, Lane M, Russell DL, Kind KL, Thompson JG. Oxygen concentration during mouse oocyte *in vitro* maturation affects embryo and fetal development. *Hum Reprod* 2007;**22**:2768–2775.

Barker DJ. The origins of the developmental origins theory. *J Intern Med* 2007; **261**:412–417.

Barker DJ, Godfrey KM, Osmond C, Bull A. The relation of fetal length, ponderal index and head circumference to blood pressure and the risk of hypertension in adult life. *Paediatr Perinat Epidemiol* 1992;**6**:35–44.

Bauer MK, Harding JE, Bassett NS, Breier BH, Oliver MH, Gallaher BH, Evans PC, Woodall SM, Gluckman PD. Fetal growth and placental function. *Mol Cell Endocrinol* 1998;**140**:115–120.

Bavister B. Oxygen concentration and preimplantation development. *Reprod Biomed Online* 2004;**9**:484–486.

Bazer FW, Spencer TE, Johnson GA, Burghardt RC. Uterine receptivity to implantation of blastocysts in mammals. *Front Biosci* 2011;**3**:745–767.

Bertolini M, Mason JB, Beam SW, Carneiro GF, Sween ML, Kominek DJ, Moyer AL, Famula TR, Sainz RD, Anderson GB. Morphology and morphometry of *in vivo*- and *in vitro*-produced bovine concepti from early pregnancy to term and association with high birth weights. *Theriogenology* 2002;**58**:973–994.

Bertolini M, Moyer AL, Mason JB, Batchelder CA, Hoffert KA, Bertolini LR, Carneiro GF, Cargill SL, Famula TR, Calvert CC *et al*. Evidence of increased substrate availability to *in vitro*-derived bovine foetuses and association with accelerated conceptus growth. *Reproduction* 2004;**128**:341–354.

Bettio D, Venci A, Levi Setti PE. Chromosomal abnormalities in miscarriages after different assisted reproduction procedures. *Placenta* 2008;**29**:S126–S128.

Blake DA, Farquhar CM, Johnson N, Proctor M. Cleavage stage versus blastocyst stage embryo transfer in assisted conception. *Cochrane Database Syst Rev* 2007; **17**:CD002118.

Bloise E, Lin W, Liu X, Simbulan R, Kolahi KS, Petraglia F, Maltepe E, Donjacour A, Rinaudo P. Impaired placental nutrient transport in mice generated by *in vitro* fertilization. *Endocrinology* 2012;**153**:3457–3467.

Bontekoe S, Blake D, Heineman MJ, Williams EC, Johnson N. Adherence compounds in embryo transfer media for assisted reproductive technologies. *Cochrane Database Syst Rev* 2010;CD007421.

Borghol N, Lornage J, Blachère T, Sophie Garret A, Lefèvre A. Epigenetic status of the H19 locus in human oocytes following *in vitro* maturation. *Genomics* 2006; **87**:417–426.

Brinster RL. Studies on the development of mouse embryos *in vitro*. IV. Interaction of energy sources. *J Reprod Fertil* 1965;**10**:227–240.

Brown S. Miscarriage and its associations. *Semin Reprod Med* 2008;**26**:391–400.

Cai LY, Izumi S, Koido S, Uchida N, Suzuki T, Matsubayashi H, Sugi T, Shida N, Kikuchi K, Yoshikata K. Abnormal placental cord insertion may induce intrauterine growth restriction in IVF-twin pregnancies. *Hum Reprod* 2006;**21**:1285–1290.

Campos DB, Papa PC, Marques JE Jr, Garbelotti F, Fátima LA, Artoni LP, Birgel EH Jr, Meirelles FV, Buratini J Jr, Leiser R *et al*. Somatic cell nuclear transfer is associated with altered expression of angiogenic factor systems in bovine placentomes at term. *Genet Mol Res* 2010;**9**:309–323.

Catt JW, Henman M. Toxic effects of oxygen on human embryo development. *Hum Reprod* 2000;**15**:199–206.

Ceelen M, van Weissenbruch MM, Vermeiden JP, van Leeuwen FE, Delemarre-van de Waal HA. Growth and development of children born after *in vitro* fertilization. *Fertil Steril* 2008;**90**:1662–1673.

Chavatte-Palmer P, Heyman Y, Richard C, Monget P, LeBourhis D, Kann G, Chilliard Y, Vignon X, Renard JP. Clinical, hormonal, and hematologic characteristics of bovine calves derived from nuclei from somatic cells. *Biol Reprod* 2002;**66**:1596–1603.

Chavatte-Palmer P, Camous S, Jammes H, Le Cleac'h N, Guillomot M, Lee RS. Review: Placental perturbations induce the developmental abnormalities often observed in bovine somatic cell nuclear transfer. *Placenta* 2012;**33**:S99–S104.

Chen XK, Wen SW, Bottomley J, Smith GN, Leader A, Walker MC. *In vitro* fertilization is associated with an increased risk for preeclampsia. *Hypertens Pregnancy* 2009; **28**:1–12.

Cleal JK, Lewis RM. The mechanisms and regulation of placental amino acid transport to the human foetus. *J Neuroendocrinol* 2008;**20**:419–426.

Coan PM, Angiolini E, Sandovici I, Burton GJ, Constância M, Fowden AL. Adaptations in placental nutrient transfer capacity to meet fetal growth demands depend on placental size in mice. *J Physiol* 2008;**586**:4567–4576.

Collier AC, Miyagi SJ, Yamauchi Y, Ward MA. Assisted reproduction technologies impair placental steroid metabolism. *J Steroid Biochem Mol Biol* 2009;**116**:21–28.

Constant F, Guillomot M, Heyman Y, Vignon X, Laigre P, Servely JL, Renard JP, Chavatte-Palmer P. Large offspring or large placenta syndrome? Morphometric analysis of late gestation bovine placentomes from somatic nuclear transfer pregnancies complicated by hydrallantois. *Biol Reprod* 2006;**75**:122–130.

Daniel Y, Schreiber L, Geva E, Amit A, Pausner D, Kupferminc MJ, Lessing JB. Do placentae of term singleton pregnancies obtained by assisted reproductive technologies differ from those of spontaneously conceived pregnancies? *Hum Reprod* 1999;**14**:1107–1110.

Dar S, Lazer T, Shah PS, Librach CL. Neonatal outcomes among singleton births after blastocyst versus cleavage stage embryo transfer: a systematic review and meta-analysis. *Hum Reprod Update* 2014;**20**:439–448.

Davies MJ, Moore VM, Willson KJ, Van Essen P, Priest K, Scott H, Haan EA, Chan A. Reproductive technologies and the risk of birth defects. *N Engl J Med* 2012; **366**:1803–1813.

de Boo HA, Harding JE. The developmental origins of adult disease (Barker) hypothesis. *Aust N Z J Obstet Gynaecol* 2006;**46**:4–14.

Delle Piane L, Lin W, Liu X, Donjacour A, Minasi P, Revelli A, Maltepe E, Rinaudo PF. Effect of the method of conception and embryo transfer procedure on mid-gestation placenta and fetal development in an IVF mouse model. *Hum Reprod* 2010;**25**:2039–2046.

Denomme MM, Zhang L, Mann MR. Embryonic imprinting perturbations do not originate from superovulation-induced defects in DNA methylation acquisition. *Fertil Steril* 2011;**96**:734–738.e2.

Desforges M, Sibley CP. Placental nutrient supply and fetal growth. *Int J Dev Biol* 2010; **54**:377–390.

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**:1526–1535.

Drost M. Complications during gestation in the cow. *Theriogenology* 2007;**68**:487–491.

Elchalal U, Ezra Y, Levi Y, Bar-Oz B, Yanai N, Intrator O, Nadjari M. Sonographically thick placenta: a marker for increased perinatal risk—a prospective cross-sectional study. *Placenta* 2000;**21**:268–272.

Esh-Broder E, Ariel I, Abas-Bashir N, Bdolah Y, Celnikier DH. 2011 Placenta accreta is associated with IVF pregnancies: a retrospective chart review. *BJOG* 2011; **118**:1084–1089.

Farin PW, Crosier AE, Farin CE. Influence of *in vitro* systems on embryo survival and fetal development in cattle. *Theriogenology* 2001;**55**:151–170.

Farin PW, Piedrahita JA, Farin CE. Errors in development of fetuses and placentas from *in vitro*-produced bovine embryos. *Theriogenology* 2006;**65**:178–191.

Fauque P, Mondon F, Letourneur F, Ripoche MA, Journot L, Barbaux S, Dandolo L, Patrat C, Wolf JP, Jouannet P *et al*. *In vitro* fertilization and embryo culture strongly impact the placental transcriptome in the mouse model. *PLoS One* 2010;**5**:e9218.

Fauser BC, Devroey P. Reproductive biology and IVF: ovarian stimulation and luteal phase consequences. *Trends Endocrinol Metab* 2003;**14**:236–242.

Feil D, Lane M, Roberts CT, Kelley RL, Edwards LJ, Thompson JG, Kind KL. Effect of culturing mouse embryos under different oxygen concentrations on subsequent fetal and placental development. *J Physiol* 2006;**572**:87–96.

Feuer SK, Rinaudo PF. Preimplantation Stress and Development. *Birth Defects Res C Embryo Today* 2012;**96**:299–214.

Feuer SK, Camarano L, Rinaudo PF. ART and health: clinical outcomes and insights on molecular mechanisms from rodent studies. *Mol Hum Reprod* 2013;**19**:189–204.

Fowden AL, Sibley C, Reik W, Constancia M. Imprinted genes, placental development and fetal growth. *Horm Res* 2006;**65**:S50–S58.

Fujii T, Moriyasu S, Hirayama H, Hashizume T, Sawai K. Aberrant expression patterns of genes involved in segregation of inner cell mass and trophectoderm lineages in bovine embryos derived from somatic cell nuclear transfer. *Cell Reprogram* 2010;**12**:617–625.

Gad A, Besenfelder U, Rings F, Ghanem N, Salilew-Wondim D, Hossain MM, Tesfaye D, Lonergan P, Becker A, Cinar U *et al*. Effect of reproductive tract environment following controlled ovarian hyperstimulation treatment on embryo development and global transcriptome profile of blastocysts: implications for animal breeding and human assisted reproduction. *Hum Reprod* 2011;**26**:1693–1707.

Gardner DK, Leese HJ. The role of glucose and pyruvate transport in regulating nutrient utilization by preimplantation mouse embryos. *Development* 1988;**104**:423–429.

Genbacev O, Zhou Y, Ludlow JW, Fisher SJ. Regulation of human placental development by oxygen tension. *Science* 1997;**277**:1669–1672.

Giritharan G, Talbi S, Donjacour A, Di Sebastiano F, Dobson AT, Rinaudo PF. Effect of *in vitro* fertilization on gene expression and development of mouse preimplantation embryos. *Reproduction* 2007;**134**:63–72.

Giritharan G, Li MW, De Sebastiano F, Esteban FJ, Horcajadas JA, Lloyd KC, Donjacour A, Maltepe E, Rinaudo PF. Effect of ICSI on gene expression and development of mouse preimplantation embryos. *Hum Reprod* 2010;**25**:3012–3024.

Giritharan G, Delle Piane L, Donjacour A, Esteban FJ, Horcajadas JA, Maltepe E, Rinaudo P. *In vitro* culture of mouse embryos reduces differential gene expression between inner cell mass and trophectoderm. *Reprod Sci* 2012;**19**:243–252.

Glazier JD, Cetin I, Perugino G, Ronzoni S, Grey AM, Mahendran D, Marconi AM, Pardi G, Sibley CP. Association between the activity of the system A amino acid transporter in the microvillous plasma membrane of the human placenta and severity of fetal compromise in intrauterine growth restriction. *Pediatr Res* 1997;**42**:514–519.

Gnarra JR, Ward JM, Porter FD, Wagner JR, Devor DE, Grinberg A, Emmert-Buck MR, Westphal H, Klausner RD, Linehan WM. Defective placental vasculogenesis causes embryonic lethality in VHL-deficient mice. *Proc Natl Acad Sci USA* 1997;**94**:9102–9107.

Gomes Sobrinho DB, Oliveira JB, Petersen CG, Mauri AL, Silva LF, Massaro FC, Baruffi RL, Cavagna M, Franco JG Jr. IVF/ICSI outcomes after culture of human embryos at low oxygen tension: a meta-analysis. *Reprod Biol Endocrinol* 2011;**9**:143.

Grazul-Bilska AT, Johnson ML, Borowicz PP, Baranko L, Redmer DA, Reynolds LP. Placental development during early pregnancy in sheep: effects of embryo origin on fetal and placental growth and global methylation. *Theriogenology* 2013;**79**:94–102.

Gunby J, Bissonnette F, Librach C, Cowan L; IVF Directors Group of the Canadian Fertility and Andrology Society. Assisted reproductive technologies (ART) in Canada: 2006 results from the Canadian ART Register. *Fertil Steril* 2010;**93**:2189–2201.

Gunby J, Bissonnette F, Librach C, Cowan L; IVF Directors Group of the Canadian Fertility and Andrology Society. Assisted reproductive technologies (ART) in Canada: 2007 results from the Canadian ART Register. *Fertil Steril* 2011;**95**:542–7.e1–10.

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**:576–582.

Hansen M, Kurinczuk JJ, Milne E, de Klerk N, Bower C. Assisted reproductive technology and birth defects: a systematic review and meta-analysis. *Hum Reprod Update* 2013;**19**:330–353.

Harrington B, Glazier J, D'Souza S, Sibley C. System A amino acid transporter activity in human placental microvillous membrane vesicles in relation to various anthropometric measurements in appropriate and small for gestational age babies. *Pediatr Res* 1999;**45**:810–814.

Helmerhorst FM, Perquin DA, Donker D, Keirse MJ. Perinatal outcome of singletons and twins after assisted conception: a systematic review of controlled studies. *BMJ* 2004;**328**:261.

Hemkemeyer SA, Schwarzer C, Boiani M, Ehmcke J, Le Gac S, Schlatt S, Nordhoff V. Effects of embryo culture media do not persist after implantation: a histological study in mice. *Hum Reprod* 2014;**29**:220–233.

Hemmings KE, Leese HJ, Picton HM. Amino acid turnover by bovine oocytes provides an index of oocyte developmental competence *in vitro*. *Biol Reprod* 2012;**86**:165, 1–12.

Hemmings KE, Maruthini D, Vyjayanthi S, Hogg JE, Balen AH, Campbell BK, Leese HJ, Picton HM. Amino acid turnover by human oocytes is influenced by gamete developmental competence, patient characteristics and gonadotrophin treatment. *Hum Reprod* 2013;**28**:1031–1044.

Hentemann M, Mousavi K, Bertheussen K. Differential pH in embryo culture. *Fertil Steril* 2011;**95**:1291–1294.

Hill JR, Burghardt RC, Jones K, Long CR, Looney CR, Shin T, Spencer TE, Thompson JA, Winger QA, Westhusin ME. Evidence for placental abnormality as the major cause of mortality in first-trimester somatic cell cloned bovine fetuses. *Biol Reprod* 2000;**63**:1787–1794.

Hill MJ, Richter KS, Heitmann RJ, Graham JR, Tucker MJ, DeCherney AH, Browne PE, Levens ED. Trophectoderm grade predicts outcomes of single-blastocyst transfers. *Fertil Steril* 2013;**99**:1283–1289.e1.

Hirayama H, Sawai K, Hirayama M, Hirai T, Kageyama S, Onoe S, Minamihashi A, Moriyasu S. Prepartum maternal plasma glucose concentrations and placental glucose transporter mRNA expression in cows carrying somatic cell clone fetuses. *J Reprod Dev* 2011;**57**:57–61.

Hoffert-Goeres KA, Batchelder CA, Bertolini M, Moyer AL, Famula TR, Anderson GB. Angiogenesis in day-30 bovine pregnancies derived from nuclear transfer. *Cloning Stem Cells* 2007;**9**:595–607.

Horcajadas JA, Mínguez P, Dopazo J, Esteban FJ, Domínguez F, Giudice LC, Pellicer A, Simón C. Controlled ovarian stimulation induces a functional genomic delay of the endometrium with potential clinical implications. *J Clin Endocrinol Metab* 2008;**93**:4500–4510.

Houghton FD. Energy metabolism of the inner cell mass and trophectoderm of the mouse blastocyst. *Differentiation* 2006;**74**:11–18.

Huang JC, Lei ZL, Shi LH, Miao YL, Yang JW, Ouyang YC, Sun QY, Chen DY. Comparison of histone modifications in *in vivo* and *in vitro* fertilization mouse embryos. *Biochem Biophys Res Commun* 2007;**354**:77–83.

International Committee for Monitoring Assisted Reproductive Technology (ICMART), European Society of Human Reproduction and Embryology Annual Meeting, 2012, Istanbul, Turkey.

Jackson RA, Gibson KA, Wu YW, Croughan MS. Perinatal outcomes in singletons following *in vitro* fertilization: a meta-analysis. *Obstet Gynecol* 2004;**103**:551–563.

Jansson T, Ylvén K, Wennergren M, Powell TL. Glucose transport and system A activity in syncytiotrophoblast microvillous and basal plasma membranes in intrauterine growth restriction. *Placenta* 2002;**23**:392–399.

Jimenez-Chillaron JC, Hernandez-Valencia M, Lightner A, Faucette RR, Reamer C, Przybyla R, Ruest S, Barry K, Otis JP, Patti ME. Reductions in caloric intake and early postnatal growth prevent glucose intolerance and obesity associated with low birthweight. *Diabetologia* 2006;**49**:1974–1984.

Jones HN, Woollett LA, Barbour N, Prasad PD, Powell TL, Jansson T. High-fat diet before and during pregnancy causes marked up-regulation of placental nutrient transport and fetal overgrowth in C57/BL6 mice. *FASEB J* 2009;**23**:271–278.

Joy J, Gannon C, McClure N, Cooke I. Is assisted reproduction associated with abnormal placentation? *Pediatr Dev Pathol* 2012;**15**:306–314.

Jurkovic D, Overton C, Bender-Atik R. Diagnosis and management of first trimester miscarriage. *BMJ* 2013;**346**:f3676.

Källén B, Finnström O, Lindam A, Nilsson E, Nygren KG, Olausson PO. Blastocyst versus cleavage stage transfer in *in vitro* fertilization: differences in neonatal outcome? *Fertil Steril* 2010;**94**:1680–1683.

Kea B, Gebhardt J, Watt J, Westphal LM, Lathi RB, Milki AA, Behr B. Effect of reduced oxygen concentrations on the outcome of *in vitro* fertilization. *Fertil Steril* 2007;**87**:213–216.

Keniry A, Oxley D, Monnier P, Kyba M, Dandolo L, Smits G, Reik W. The H19 lincRNA is a developmental reservoir of miR-675 that suppresses growth and Igf1r. *Nat Cell Biol* 2012;**14**:659–665.

King KK, Seidel GE Jr, Elsden RP. Bovine embryo transfer pregnancies. I. Abortion rates and characteristics of calves. *J Anim Sci* 1985;**61**:747–757.

Kolahi KS, Donjacour A, Liu X, Lin W, Simbulan RK, Bloise E, Maltepe E, Rinaudo P. Effect of substrate stiffness on early mouse embryo development. *PLoS One* 2012; **7**:e41717.

Kovacic B, Sajko MC, Vlaisavljević V. A prospective, randomized trial on the effect of atmospheric versus reduced oxygen concentration on the outcome of intracytoplasmic sperm injection cycles. *Fertil Steril* 2010;**94**:511–519.

Krisher RL, Prather RS. A role for the Warburg effect in preimplantation embryo development: metabolic modification to support rapid cell proliferation. *Mol Reprod Dev* 2012;**79**:311–320.

Kruip ThAM, den Daas JHG. *In vitro* produced and cloned embryos: effects on pregnancy, parturition and offspring. *Theriogenology* 1997;**47**:43–52.

Lalosević D, Tabs D, Krnojelac D, Vejnović T, Radunović N. Histological characteristics of placentas from assisted reproduction programs. *Med Pregl* 2003;**56**:521–527.

Le F, Wang LY, Wang N, Li L, Li le J, Zheng YM, Lou HY, Liu XZ, Xu XR, Sheng JZ *et al. In vitro* fertilization alters growth and expression of Igf2/H19 and their epigenetic mechanisms in the liver and skeletal muscle of newborn and elder mice. *Biol Reprod* 2013;**88**:75.

Leese HJ. Metabolism of the preimplantation embryo: 40 years on. *Reproduction* 2012; **143**:417–427.

Mahendran D, Donnai P, Glazier JD, D'Souza SW, Boyd RD, Sibley CP. Amino acid (system A) transporter activity in microvillous membrane vesicles from the placentas of appropriate and small for gestational age babies. *Pediatr Res* 1993; **34**:661–665.

Maheshwari A, Kalampokas T, Davidson J, Bhattacharya S. Obstetric and perinatal outcomes in singleton pregnancies resulting from the transfer of blastocyst-stage versus cleavage-stage embryos generated through *in vitro* fertilization treatment: a systematic review and meta-analysis. *Fertil Steril* 2013;**100**:1615–21.e1–10.

Mäkinen S, Söderström-Anttila V, Vainio J, Suikkari AM, Tuuri T. Does long *in vitro* culture promote large for gestational age babies? *Hum Reprod* 2013;**28**:828–834.

Malassiné A, Frendo JL, Evain-Brion D. A comparison of placental development and endocrine functions between the human and mouse model. *Hum Reprod Update* 2003;**9**:531–539.

Mamo S, Mehta JP, McGettigan P, Fair T, Spencer TE, Bazer FW, Lonergan P. RNA sequencing reveals novel gene clusters in bovine conceptuses associated with maternal recognition of pregnancy and implantation. *Biol Reprod* 2011;**85**:1143–1151.

Manipalviratn S, DeCherney A, Segars J. Imprinting disorders and assisted reproductive technology. *Fertil Steril* 2009;**91**:305–315.

Mann MR, Lee SS, Doherty AS, Verona RI, Nolen LD, Schultz RM, Bartolomei MS. Selective loss of imprinting in the placenta following preimplantation development in culture. *Development* 2004;**131**:3727–3735.

Marchesi DE, Qiao J, Feng HL. Embryo manipulation and imprinting. *Semin Reprod Med* 2012;**30**:323–334.

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.

Martyn CN, Barker DJ, Osmond C. Mothers' pelvic size, fetal growth, and death from stroke and coronary heart disease in men in the UK. *Lancet* 1996;**348**:1264–1268.

McMillan WH, Peterson AJ, Hall DRH, Donnison MJ. Embryo and recipient contributions to embryo loss to day 60 in heifers receiving either one or two *in vitro*-produced embryos. *Theriogenology* 1997;**47**:370–370.

McNeill RE, Sreenan JM, Diskin MG, Cairns MT, Fitzpatrick R, Smith TJ, Morris DG. Effect of systemic progesterone concentration on the expression of progesterone-responsive genes in the bovine endometrium during the early luteal phase. *Reprod Fertil Dev* 2006;**18**:573–583.

Meas T. Fetal origins of insulin resistance and the metabolic syndrome: a key role for adipose tissue? *Diabetes Metab* 2010;**36**:11–20.

Meegdes BH, Ingenhoes R, Peeters LL, Exalto N. Early pregnancy wastage: relationship between chorionic vascularization and embryonic development. *Fertil Steril* 1988; **49**:216–220.

Meintjes M, Chantilis SJ, Douglas JD, Rodriguez AJ, Guerami AR, Bookout DM, Barnett BD, Madden JD. A controlled randomized trial evaluating the effect of lowered incubator oxygen tension on live births in a predominantly blastocyst transfer program. *Hum Reprod* 2009;**24**:300–307.

Miles JR, Farin CE, Rodriguez KF, Alexander JE, Farin PW. Effects of embryo culture on angiogenesis and morphometry of bovine placentas during early gestation. *Biol Reprod* 2005;**73**:663–671.

Milki AA, Jun SH, Hinckley MD, Behr B, Giudice LC, Westphal LM. Incidence of monozygotic twinning with blastocyst transfer compared to cleavage-stage transfer. *Fertil Steril* 2003;**79**:503–506.

Murray MK, DeSouza MM, Messinger SM. Oviduct during early pregnancy: hormonal regulation and interactions with the fertilized ovum. *Microsc Res Tech* 1995; **31**:497–506.

Nelissen EC, Dumoulin JC, Daunay A, Evers JL, Tost J, van Montfoort AP. Placentas from pregnancies conceived by IVF/ICSI have a reduced DNA methylation level at the H19 and MEST differentially methylated regions. *Hum Reprod* 2013; **28**:1117–1126.

Nelson DM, Smith SD, Furesz TC, Sadovsky Y, Ganapathy V, Parvin CA, Smith CH. Hypoxia reduces expression and function of system A amino acid transporters in cultured term human trophoblasts. *Am J Physiol Cell Physiol* 2003;**284**:C310–C315.

Norwitz ER. Defective implantation and placentation: laying the blueprint for pregnancy complications. *Reprod Biomed Online* 2006;**13**:591–599.

Ozawa M, Sakatani M, Yao J, Shanker S, Yu F, Yamashita R, Wakabayashi S, Nakai K, Dobbs KB, Sudano MJ *et al.* Global gene expression of the inner cell mass and trophectoderm of the bovine blastocyst. *BMC Dev Biol* 2012;**12**:33.

Pace MM, Augenstein ML, Betthauser JM, Childs LA, Eilertsen KJ, Enos JM, Forsberg EJ, Golueke PJ, Graber DF, Kemper JC *et al.* Ontogeny of cloned cattle to lactation. *Biol Reprod* 2002;**67**:334–339.

Papanikolaou EG, Bourgain C, Kolibianakis E, Tournaye H, Devroey P. Steroid receptor expression in late follicular phase endometrium in GnRH antagonist IVF cycles is already altered, indicating initiation of early luteal phase transformation in the absence of secretory changes. *Hum Reprod* 2005;**20**:1541–1547.

Papanikolaou EG, Camus M, Kolibianakis EM, Van Landuyt L, Van Steirteghem A, Devroey P. *In vitro* fertilization with single blastocyst-stage versus single cleavage-stage embryos. *N Engl J Med* 2006;**354**:1139–1146.

Papanikolaou EG, Kolibianakis EM, Tournaye H, Venetis CA, Fatemi H, Tarlatzis B, Devroey P. Live birth rates after transfer of equal number of blastocysts or cleavage-stage embryos in IVF. A systematic review and meta-analysis. *Hum Reprod* 2008;**23**:91–99.

Phipps K, Barker DJ, Hales CN, Fall CH, Osmond C, Clark PM. Fetal growth and impaired glucose tolerance in men and women. *Diabetologia* 1993;**36**:225–228.

Power MA, Tam PP. Onset of gastrulation, morphogenesis and somitogenesis in mouse embryos displaying compensatory growth. *Anat Embryol* 1993;**187**:493–504.

Prather RS, First NL. A review of early mouse embryogenesis and its applications to domestic species. *J Anim Sci* 1988;**66**:2626–2635.

Ptak GE, D'Agostino A, Toschi P, Fidanza A, Zacchini F, Czernik M, Monaco F, Loi P. Post-implantation mortality of *in vitro* produced embryos is associated with DNA methyltransferase 1 dysfunction in sheep placenta. *Hum Reprod* 2013;**28**:298–305.

Raunig JM, Yamauchi Y, Ward MA, Collier AC. Assisted reproduction technologies alter steroid delivery to the mouse fetus during pregnancy. *J Steroid Biochem Mol Biol* 2011a;**126**:26–34.

Raunig JM, Yamauchi Y, Ward MA, Collier AC. Placental inflammation and oxidative stress in the mouse model of assisted reproduction. *Placenta* 2011b;**32**:852–858.

Redel BK, Brown AN, Spate LD, Whitworth KM, Green JA, Prather RS. Glycolysis in preimplantation development is partially controlled by the Warburg Effect. *Mol Reprod Dev* 2012;**79**:262–271.

Red-Horse K, Zhou Y, Genbacev O, Prakobphol A, Foulk R, McMaster M, Fisher SJ. Trophoblast differentiation during embryo implantation and formation of the maternal-fetal interface. *J Clin Invest* 2004;**114**:744–754.

Richardson MK, Hanken J, Gooneratne ML, Pieau C, Raynaud A, Selwood L, Wright GM. There is no highly conserved embryonic stage in the vertebrates: implications for current theories of evolution and development. *Anat Embryol* 1997;**196**:91–106.

Rinaudo PF, Lamb J. Fetal origins of perinatal morbidity and/or adult disease. *Semin Reprod Med* 2008;**26**:436–445.

Rinaudo P, Schultz RM. Effects of embryo culture on global pattern of gene expression in preimplantation mouse embryos. *Reproduction* 2004;**128**:301–311.

Rinaudo P, Wang E. Fetal programming and metabolic syndrome. *Annu Rev Physiol* 2012; **74**:107–130.

Rinaudo PF, Giritharan G, Talbi S, Dobson AT, Schultz RM. Effects of oxygen tension on gene expression in preimplantation mouse embryos. *Fertil Steril* 2006;**86**:1252–1265, 1265.e1–36.

Romundstad LB, Romundstad PR, Sunde A, von Düring V, Skjaerven R, Vatten LJ. Increased risk of placenta previa in pregnancies following IVF/ICSI; a comparison of ART and non-ART pregnancies in the same mother. *Hum Reprod* 2006;**21**:2353–2358.

Romundstad LB, Romundstad PR, Sunde A, von Düring V, Skjaerven R, Gunnell D, Vatten LJ. Effects of technology or maternal factors on perinatal outcome after assisted fertilisation: a population-based cohort study. *Lancet* 2008;**372**:737–743.

Rutherford JN. Fetal signaling through placental structure and endocrine function: illustrations and implications from a nonhuman primate model. *Am J Hum Biol* 2009;**21**:745–753.

Salilew-Wondim D, Tesfaye D, Hossain M, Held E, Rings F, Tholen E, Looft C, Cinar U, Schellander K, Hoelker M. Aberrant placenta gene expression pattern in bovine pregnancies established after transfer of cloned or *in vitro* produced embryos. *Physiol Genomics* 2013;**45**:28–46.

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.

Schieve LA, Meikle SF, Ferre C, Peterson HB, Jeng G, Wilcox LS. Low and very low birth weight in infants conceived with use of assisted reproductive technology. *N Engl J Med* 2002;**346**:731–737.

Schwarzer C, Esteves TC, Araúzo-Bravo MJ, Le Gac S, Nordhoff V, Schlatt S, Boiani M. ART culture conditions change the probability of mouse embryo gestation through defined cellular and molecular responses. *Hum Reprod* 2012; **27**:2627–2640.

Seckl JR, Holmes MC. Mechanisms of disease: glucocorticoids, their placental metabolism and fetal 'programming' of adult pathophysiology. *Nat Clin Pract Endocrinol Metab* 2007;**3**:479–488.

Simon C, Domínguez F, Valbuena D, Pellicer A. 2003 The role of estrogen in uterine receptivity and blastocyst implantation. *Trends Endocrinol Metab* 2003;**14**:197–199.

Sinclair KD, Young LE, Wilmut I, McEvoy TG. In-utero overgrowth in ruminants following embryo culture: lessons from mice and a warning to men. *Hum Reprod* 2000;**155**:68–86.

Sousa LM, Campos DB, Fonseca VU, Viau P, Kfoury JR Jr, Oliveira CA, Binelli M, Buratini J Jr, Papa PC. Vascular endothelial growth factor A (VEGFA) modulates bovine placenta steroidogenesis *in vitro*. *Placenta* 2012;**33**:788–794.

Templeton A. Infertility and the establishment of pregnancy—overview. *Br Med Bull* 2000;**56**:577–587.

Tummers P, De Sutter P, Dhont M. Risk of spontaneous abortion in singleton and twin pregnancies after IVF/ICSI. *Hum Reprod* 2003;**18**:1720–1723.

Tunster SJ, Jensen AB, John RM. Imprinted genes in mouse placental development and the regulation of fetal energy stores. *Reproduction* 2013;**145**:R117–R137.

Van Soom A, Boerjan ML, Bols PE, Vanroose G, Lein A, Coryn M, de Kruif A. Timing of compaction and inner cell allocation in bovine embryos produced *in vivo* after superovulation. *Biol Reprod* 1997;**57**:1041–1049.

Waldenström U, Engström AB, Hellberg D, Nilsson S. Low-oxygen compared with high-oxygen atmosphere in blastocyst culture, a prospective randomized study. *Fertil Steril* 2009;**91**:2461–2465.

Walker SK, Hartwich KM, Robinson JS. Long-term effects on offspring of exposure of oocytes and embryos to chemical and physical agents. *Hum Reprod Update* 2000; **6**:564–577.

Wang JX, Norman RJ, Wilcox AJ. Incidence of spontaneous abortion among pregnancies produced by assisted reproductive technology. *Hum Reprod* 2004; **19**:272–277.

Watanabe S, Nagai T. Survival of embryos and calves derived from somatic cell nuclear transfer in cattle: a nationwide survey in Japan. *Anim Sci J* 2011;**82**:360–365.

Young LE, Sinclair KD, Wilmut I. Large offspring syndrome in cattle and sheep. *Rev Reprod* 1998;**3**:155–163.

Zhang Y, Zhang YL, Feng C, Wu YT, Liu AX, Sheng JZ, Cai J, Huang HF. Comparative proteomic analysis of human placenta derived from assisted reproductive technology. *Proteomics* 2008;**8**:4344–4356.

Zhang Y, Cui Y, Zhou Z, Sha J, Li Y, Liu J. Altered global gene expressions of human placentae subjected to assisted reproductive technology treatments. *Placenta* 2010;**31**:251–258.

Zhang Y, Zhao W, Jiang Y, Zhang R, Wang J, Li C, Zhao H, Gao L, Cui Y, Zhou Z *et al.* Ultrastructural study on human placentae from women subjected to assisted reproductive technology treatments. *Biol Reprod* 2011;**85**:635–642.

Zhu J, Lin S, Li M, Chen L, Lian Y, Liu P, Qiao J. Effect of *in vitro* culture period on birthweight of singleton newborns. *Hum Reprod* 2014;**29**:448–454.