Advanced maternal age causes adverse programming of mouse blastocysts leading to altered growth and impaired cardiometabolic health in post-natal life

M.A. Velazquez¹,², C.G.C. Smith¹, N.R. Smyth¹, C. Osmond³, and T.P. Fleming¹,*

¹Centre for Biological Sciences, University of Southampton, Southampton SO16 6YD, UK ²School of Agriculture, Food & Rural Development, Newcastle University, Newcastle Upon Tyne NE1 7RU, UK ³MRC Lifecourse Epidemiology Unit, University of Southampton, Southampton SO16 6YD, UK

*Correspondence address. Tel: + 44-2381-204145; E-mail: t.p.fleming@soton.ac.uk

Submitted on December 18, 2015; resubmitted on June 8, 2016; accepted on June 17, 2016

STUDY QUESTION: Does advanced maternal age (AMA) in mice affect cardiometabolic health during post-natal life in offspring derived from an assisted reproduction technology (ART) procedure?

SUMMARY ANSWER: Offspring derived from blastocysts collected from aged female mice displayed impaired body weight gain, blood pressure, glucose metabolism and organ allometry during post-natal life compared with offspring derived from blastocysts from young females; since all blastocysts were transferred to normalized young mothers, this effect is independent of maternal pregnancy conditions.

WHAT IS KNOWN ALREADY: Although studies in mice have shown that AMA can affect body weight and behaviour of offspring derived from natural reproduction, data on the effects of AMA on offspring cardiometabolic health during post-natal development are not available. Given the increasing use of ART to alleviate infertility in women of AMA, it is pivotal to develop ART–AMA models addressing the effects of maternal aging on offspring health.

STUDY DESIGN, SIZE, DURATION: Blastocysts from old (34–39 weeks) or young (8–9 weeks) C57BL/6 females mated with young CBA males (13–15 weeks) were either subjected to differential cell staining (inner cell mass and trophectoderm) or underwent embryo transfer (ET) into young MF1 surrogates (8–9 weeks) to produce young (Young-ET, 9 litters) and old (Old-ET, 10 litters) embryo-derived offspring. Offspring health monitoring was carried out for 30 weeks.

PARTICIPANTS/MATERIALS, SETTING, METHODS: All animals were fed with standard chow. Blood pressure was measured at post-natal Weeks 9, 15 and 21, and at post-natal Week 30 a glucose tolerance test (GTT) was performed. Two days after the GTT mice were killed for organ allometry. Blastocyst cell allocation variables were evaluated by T-test and developmental data were analysed with a multi-level random effects regression model.

MAIN RESULTS AND THE ROLE OF CHANCE: The total number of cells in blastocysts from aged mice was decreased (P < 0.05) relative to young mice due to a lower number of cells in the trophectoderm (mean ± SEM: 34.5 ± 2.1 versus 29.6 ± 1.0). Weekly body weight did not differ in male offspring, but an increase in body weight from Week 13 onwards was observed in Old-ET females (final body weight at post-natal Week 30: 38.5 ± 0.8 versus 33.4 ± 0.8 g, P < 0.05). Blood pressure was increased in Old-ET offspring at Weeks 9–15 in males (Week 9: 108.5 ± 3.13 versus 100.8 ± 1.5 mmHg, Week 15: 112.9 ± 3.2 versus 103.4 ± 2.1 mmHg) and Week 15 in females (115.9 ± 3.7 versus 102.8 ± 0.7 mmHg, all P < 0.05 versus Young-ET). The GTT results and organ allometry were not affected in male offspring. In contrast, Old-ET females displayed a greater (P < 0.05) peak glucose concentration at 30 min during the GTT (21.1 ± 0.4 versus 17.8 ± 1.16 mmol/l) and their spleen weight (88.2 ± 2.6 ± 105.1 ± 4.6 mg) and several organ:body weight ratios (g/g × 10³) were decreased (P < 0.05 versus Young-ET), including the heart (3.7 ± 0.06 versus 4.4 ± 0.08), lungs (4.4 ± 0.1 versus 5.0 ± 0.1), spleen (2.4 ± 0.06 versus 3.2 ± 0.1) and liver (36.4 ± 0.6 versus 39.1 ± 0.9).

LIMITATIONS, REASONS FOR CAUTION: Results from experimental animal models cannot be extrapolated to humans. Nevertheless, they are valuable to develop conceptual models that can produce hypotheses for eventual testing in the target species (i.e. humans).
The text contains a study on the effects of advanced maternal age (AMA) on offspring during post-natal development. It discusses the increased risk of congenital abnormalities, behavior, and cognitive outcomes associated with AMA. The study highlights the importance of experimental animal models in understanding the long-term effects of AMA on metabolic and cardiovascular function. It also mentions the increasing trend in the use of assisted reproduction technology (ART) and the need to develop experimental ART models of AMA to better understand its effects. The key points include:

- Advanced maternal age (AMA) is a risk factor for congenital abnormalities and behavior and cognitive outcomes in children.
- AMA may decrease the lifespan and exert a positive effect on some behavioral and cognitive outcomes.
- Experimental work in mice indicated that AMA can have a long-term effect on the metabolic and cardiovascular function of offspring.
- Further research is needed to understand the long-term effects of AMA on offspring.
preimplantation embryo itself from those that may derive from the maternal tract and systemic environment of the pregnancy following embryo transfer (ET).

In the present study, using a murine ET model, we examine the effects of AMA on the post-natal development of offspring under an ART-derived procedure. To avoid potential adverse programming from ovulation induction, IVF and extended embryo culture, and paternal conditions (Watkins et al., 2007; El Hajj and Haaf, 2013) and to focus attention on outcomes mainly related to maternal age of embryos, we used aged or young females naturally mated to young males, with subsequent collection and immediate transfer of blastocysts to young recipients. We provide evidence that offspring of aged mice display alterations in body weight gain, blood pressure, glucose metabolism and organ allometry during post-natal development that predominantly affect females. Furthermore, our data demonstrate that these adverse phenotypes are already programmed by the blastocyst stage.

Materials and Methods

Animals

All animal experimentation was conducted in accordance with the UK Home Office Animal (Scientific Procedures) Act 1986 and local ethics committee at the University of Southampton. All animals were bred in-house (University of Southampton, Biomedical Research Facility) and kept on a 0700–1900 h light cycle at a temperature of 20–22°C. Water and standard laboratory chow (Special Diet Services, Ltd, Witham, Essex, UK) was provided ad libitum to all animals used in the study.

In vivo production of blastocysts

Non-superovulated virgin young (8–9 weeks) and old (34–39 weeks) female mice (C57BL/6) were mated with young CBA males (10–15 weeks; Fig. 1). Male–female pairs were housed overnight and the presence of a vaginal plug the following morning was regarded as a sign of successful mating. Females were considered to be on embryonic Day 0.5 (E0.5) at midday on the day the vaginal plug was detected. On E3.5 mice were killed by cervical dislocation and uterine horns were immediately dissected and placed in warm (37°C) saline solution (BR0053G, OXOID, UK). Each uterine horn was then gently flushed on an empty petri dish, under a stereomicroscope, with 1 ml of H6 medium supplemented with 4 mg/ml bovine serum albumin (BSA, A3311, Sigma, UK; Nasr-Esfahani et al., 1990). Arrested and/or fragmented embryos at the 2- to 8-cell stage were classified as degenerated embryos. Morula and blastocyst with clear signs of fragmentation were also considered degenerated embryos. All non-fragmented 1-cell structures (i.e. absence of first cleavage division) were classified as unfertilized oocytes (i.e. ova; Wu et al., 2010). All embryos in the stage of morula or blastocyst with no signs of fragmentation were classified as viable embryos. Viability (total viable embryos/total embryos × 100), fertilization (total embryos/total embryos + ova × 100) and degeneration (degenerated embryos/total embryos × 100) were assessed.

Figure 1  Murine model of AMA used in the study. Young (8–9 weeks) or old (34–39 weeks) C57BL/6 females were mated with young CBA males (10–15 weeks) to obtain young or old blastocysts (Day 3.5 of pregnancy). Blastocysts were then transferred to young MF1 recipients (8–9 weeks) to obtain young (Young-ET) or old (Old-ET) embryo-derived offspring. ET, embryo transfer.
forms and good overall quality were obtained per mouse (Williams et al., 2011). At post-natal Week 30 a standard protocol for glucose tolerance test (GTT) was performed in unrestrained conscious mice following a 15 h fasting period. Immediately after the GTT, mice were placed in clean cages with food and water ad libitum. Two days after the GTT, mice were killed by cervical dislocation and organs (i.e. spleen, liver, left and right kidneys, heart and lungs) and carcass weighed.

Differential cell staining in blastocysts

Blastocysts with a blastocoel cavity greater than half of the volume of the embryo (i.e. expanded blastocysts) were subjected to differential nuclear labelling according to the protocol developed by Hardy et al. (1989) with some modifications. Unless otherwise indicated, embryos were processed in 50 μl drops. After removing the zona pellucida in 1 ml of warm (37°C) acid Tyrode’s solution (T1788, Sigma), blastocysts were washed for 15–20 min in 1 ml H6-BSA followed by 10 min incubation in 10% trinitrobenzenesulfonic acid solution (TNBS, P-2297, Sigma) at room temperature. H6 medium supplemented with 1% polyvinylpyrrolidone (PVP, P0930, Sigma) was used to prepare the 10% TNBS solution. Blastocysts were then washed three times in H6-PVP and incubated for 10 min in 0.4 mg/ml goat anti-dinitrophenyl antibody (D9781, Sigma) in H6-PVP at room temperature. After washing three times in H6-PVP, blastocysts were incubated in 50 μl of reconstituted (1:10 dilution with H6-BSA) Low-Tox® guinea pig complement (CL4051, Cedarslane, Canada) supplemented with 4 μl propidium iodide (1 mg/ml, P4170, Sigma) for 15 min at 37°C. Blastocysts were washed again three times with H6-BSA and fixed in 1 ml ice-cold ethanol supplemented with 1% Bismesalexide H 33258 (2.5 mg/ml, B2883, Sigma) at 4°C for 1 h. For cell quantification, blastocysts were washed in 1 ml ice-cold fresh ethanol and mounted onto a glass microscope slide in a ~4 μl drop of glycerol (G5516, Sigma) and coverslipped. Digital photographs of blastocysts were obtained with an inverted epifluorescence microscope (Axiovert 200M, Carl Zeiss Ltd) in a darkened room. Cell nuclei were manually counted with the MetaMorph software (Version 6.2r6, Molecular Devices).

Embryo transfer

Blastocysts were washed three times in M2 medium before ET (Watkins et al., 2007). The uterine horn was exposed by flank laparotomy and six expanding blastocysts were transferred with a minimal amount of medium into the uterine cavity of MF1 pseudo-pregnant (E2.5) young recipients (8–9 weeks; Fig. 1). The uterine horn was then placed back into the abdominal cavity and the incision site closed. The procedure was repeated in the opposite flank where another six expanding blastocysts were transferred. Following ET, recipients were placed individually in clean cages to recover from anaesthesia in a warm room (28–30°C). Females were then moved to a quiet room where they were kept for the rest of the pregnancy and lactation.

Offspring analysis

ET-derived offspring were weaned 3 weeks after birth, separated according to sex, and their body weight recorded weekly for 30 weeks. Systolic blood pressure was measured with a standard protocol at post-natal Weeks 9, 15 and 21 by tail-cuff plethysmography with the Non-Invasive Blood Pressure Monitor (NIIBP-8, Columbus Instruments, Columbus, OH, USA) in a warm room (28–30°C; Watkins et al., 2007, 2008). Five readings with good waveforms and good overall quality were obtained per mouse (Williams et al., 2011). At post-natal Week 30 a standard protocol for glucose tolerance test (GTT) was performed in unrestrained conscious mice following a 15 h overnight fast. A blood glucose metre (Accu-Chek, Aviva, Roche Diagnostics GmbH, Germany) was used to measure glucose in small drops of blood collected by tail tipping. Twenty minutes before starting the GTT, anaesthetic cream (Lidocaine 5%, Teva, UK) was applied to the tail. After recording of the baseline glucose level (0 min), a glucose (G8270, Sigma) solution (20%, in sterile, distilled water) was administered by i.p. injection at a dose of 2 g/kg. Glucose levels were measured 15, 30, 60 and 120 min after glucose administration (Constantinou et al., 2014; Wedemann et al., 2016). Water was provided ad libitum during fasting and GTT. Immediately after the GTT, mice were placed in clean cages with food and water ad libitum. Of the five blood pressure readings taken at each time point per mouse, the lowest and highest values were discarded and the mean of the three middle values was used for statistical analysis (Williams et al., 2011). Area under the curve values were calculated for GTT data by the trapezoidal rule (Matthews et al., 1990). Data are presented as mean ± SEM unless otherwise indicated.

Results

Maternal aging can alter cell allocation of blastocysts in mice

The percentage of plug-positive females that failed to yield embryos (Fig. 2A) and the number of embryos produced per donor was not different between the age groups (Fig. 2C). However, the fertilization rate was decreased in aged females (Fig. 2B). Some of the embryos were subjected to differential cell staining and the analysis revealed a decrease in the total number of cells in blastocysts of aged mice due to a lower number of cells in the trophoderm (Fig. 3A). Other variables of cell allocation were not affected (Fig. 3B).

Offspring derived from blastocysts collected from aged mice show an increased weight gain during post-natal life in a sex-specific manner

To examine the effects of AMA on the post-natal development of offspring under an ART-derived procedure, we transferred blastocysts from old mothers into young surrogates and the resultant offspring (Old-ET) was compared with offspring derived from blastocysts collected from young mothers that were transferred into equivalent young surrogates (Young-ET; Fig. 1). Following ET, the number of pups per litter was not different between the groups (Fig. 4A), nor the efficiency of ET to yield live offspring (Old 66%; Young 65%). Similarly, the male to female proportion was not affected by origin of the embryo (Fig. 4B). Monitoring of body weight every week revealed no differences in weight gain in male offspring across treatments at any...
time point during the experimental trial (Fig. 5A). In contrast, Old-ET females were heavier than Young-ET females during most of post-natal development. The difference in body weight started to become apparent at post-natal Week 13 and remained significant for virtually the rest of the study (Fig. 5C).

**Offspring derived from blastocysts collected from aged mice develop hypertension during post-natal life in a non-sex-specific manner**

Non-invasive systolic blood pressure measurements were carried out in the offspring at post-natal Week 9, 15 and 21. The analysis showed a significant increase in blood pressure in Old-ET male offspring at Weeks 9 and 15 compared with Young-ET counterparts (Fig. 5B). However, by Week 21 systolic blood pressure values were not significantly different between the groups (Fig. 5B). A similar scenario was observed in female offspring, but the difference was only significant at post-natal Week 15 (Fig. 5D). The average of the three measurements was higher in Old-ET offspring for both sexes (Males: 106.6 ± 1.4 versus 102.6 ± 0.9 mmHg; Females: 105.0 ± 1.4 versus 101.5 ± 0.7 mmHg), but it did not reach statistical significance (P < 0.10).
Offspring derived from blastocysts collected from aged mice display altered glucose homeostasis in a sex-specific manner

Offspring were subjected to an i.p. GTT at the end of the study on post-natal Week 30. Basal glucose concentrations following overnight fasting (i.e. 0 min) were not different between the groups in both sexes (Fig. 6A and B). In male offspring, glucose levels at different time points after glucose administration and the area under the curve were not affected by the origin of the offspring (Fig. 6A). In contrast, Old-ET females showed a greater peak glucose concentration 30 min after glucose injection compared with Young-ET counterparts (Fig. 6B). However, 60 min after glucose administration the difference started to lose significance \( (P = 0.050) \), and by 120 min post glucose injection glucose levels have returned to basal values and were very similar between the groups. The area under the curve in Old-ET females (Fig. 6B) was higher compared with Young-ET counterparts, but the difference was not significant \( (P > 0.10) \).

**Offspring derived from blastocysts collected from aged mice exhibit altered organ allometry in a sex-specific manner**

Analysis of organ allometry was carried out at the end of the study on post-natal Week 30. No significant differences in carcass weight were observed in males \((38.5 \pm 0.5 \text{ and } 40.0 \pm 1.0 \text{ g for Old-ET and Young-ET offspring, respectively})\). In contrast, the carcass weight of females was higher in the Old-ET group \((32.9 \pm 0.6 \text{ versus } 29.3 \pm 0.7 \text{ g, } P < 0.05)\). In male offspring, organ weights and organ weight:body weight ratios were not different between the groups (Fig. 7A and B). However, in Old-ET females the spleen was lighter compared with Young-ET females (Fig. 7C). Furthermore, the organ weight:body weight ratio for heart, lungs, spleen and liver were decreased in Old-ET females (Fig. 7D). Most of the internal organs of female offspring were of similar weight between the groups. Hence, with the exception of the spleen, the undersized organs in Old-ET females are more related to their increased body weight.

**Discussion**

In this study, we have investigated the long-term effects of AMA on offspring development during post-natal life. Using an ET model our data show that offspring derived from embryos collected from aged female mice (mated with young males) and transferred into young embryo recipients display alterations in body weight gain, blood pressure, glucose metabolism and organ allometry during post-natal development. Most of the altered phenotypes were observed in a sex-specific manner, where females were more affected. Our study is the first to examine metabolic and cardiovascular function in offspring of aged female mice under an ART-related procedure. The fact that phenotypic alterations
were observed under a normalized maternal in vivo environment following ET indicates that some of the adverse programming induced by AMA is already established by the time the embryo reaches the blastocyst stage and independent of subsequent conditions of pregnancy. In support of our current findings, experimental evidence in rodents and ruminants has shown that altered phenotypes during post-natal life can be programmed during the periconceptional period when critical reproductive events such as ovarian folliculogenesis and/or preimplantation embryo development are exposed to undernutrition (Sinclair et al., 2007; Watkins et al., 2008), overnutrition (Rattanatray et al., 2010; Kleemann et al., 2015), ART-related procedures (Watkins et al., 2007; Rexhaj et al., 2013; Donjacour et al., 2014; López-Cardona et al., 2015) or inflammation-like events (Williams et al., 2011).

We used 34–39-week-old mice in our AMA model in an effort to reflect the onset of reproductive aging in middle-aged females rather than substantial loss of reproductive function during advanced senescence. Indeed, in our study the number of embryos recovered from non-superoovulated old mice was not affected, which is comparable with the lack of effect of AMA on litter size following natural mating reported in AMA studies with mice of similar ages (Lopes et al., 2009; Yue et al., 2012). Although cases of pregnancies in women in their 60s have been reported (Cutas and Smajdor, 2015), we believe our model is more akin with the current trend in delayed childbearing, in which middle-aged women rather than elderly individuals are more likely to delay motherhood.

Human embryos from aging mothers are known to show an increase in aneuploidy, contributing to reduced fertility with age (Munné, 2006; Jones and Lane, 2013). In comparison, depending on the mouse strain (Yun et al., 2014), murine embryos are less likely to display aneuploidy (Carbone and Chavez, 2015), especially in the age range used in the present study (Jones and Lane, 2013). This distinction, although reflecting a difference to the conditions normally encountered during human assisted conception practice, is to our advantage in the current mouse model since it permits increased survival to screen post-natal health in relation to maternal age. Indeed, it is believed that aneuploidy is the main cause of human IVF failure, resulting in implantation failure, miscarriage or birth of offspring. 

Figure 6 AMA can affect glucose metabolism in mice. Intraperitoneal GTT outcome in male (A) and female (B) offspring derived from the transfer of blastocysts collected from young (Young-ET, 9 litters) or old (Old-ET, 10 litters) mothers into young embryo recipients. n = number of animals. Multilevel random effects regression analysis. *Indicates a significant difference (P < 0.05) between the groups (Young-ET versus Old-ET).
with congenital disorders (Campbell et al., 2013; Franasiak and Scott, 2014). Moreover, from a clinical practice perspective, our model relates to conditions of human gestational surrogacy where AMA embryos are transferred to a younger recipient and the potential programming of such embryos mediated through donor maternal age.

Our results indicate that maternal aging can induce a decrease in the number of cells at the blastocyst stage, especially in the trophectoderm. A recent study in rats reported that growth-restricted females with AMA at conception produced blastocysts with decreased cell number attributed to a lower cell number in trophectoderm (Master et al., 2007). Low cell number in blastocysts has been associated with decreased placental and fetal weight in a mouse model of mitochondrial dysfunction (Wakefield et al., 2011). We did not analyse fetal development in our study, but a reduction in embryo size and somite number without alterations in litter size has been reported in a murine model of AMA (Lopes et al., 2009). In our study, birthweight was not examined, but our data suggest that deviation in cell proliferation at early stages of development is an early sign of impaired post-natal health. Indeed, rodent offspring developing high blood pressure in adulthood showed alterations in cell number at the blastocyst stage after exposure to undernutrition or to an in vitro environment during the preimplantation period (Kwong et al., 2000; Watkins et al., 2007).

The subtle impairment of glucose homeostasis found in female offspring of old mice in our study gives partial support to a recent multi-country study that suggested that AMA can induce increased plasma glucose concentrations in young adults (Fall et al., 2015). However, in that epidemiological human study the impaired glucose metabolism was not sex-specific (Fall et al., 2015), which contrast with our results. It will be interesting to test under experimental conditions if this lack of gender specificity on post-natal glucose metabolism is due to an added effect of paternal aging. Previous epidemiological human studies also failed to detect an effect of AMA on offspring body weight (Myrskyla and Fenelon, 2012) or blood pressure (Fall et al., 2015; Verroken et al., 2015) during adulthood. Here, it is important to highlight that in epidemiological studies it is extremely difficult to control relevant confounding factors and to collect accurate long-term information on lifestyle habits and health status. Under well-controlled experimental conditions in mice, we found that maternal aging can programme the development of high blood pressure in adulthood in a non-sex-specific manner. However, the high blood pressure observed in Old-ET offspring was accompanied by increased body weight only in females. Similarly, in previous experiments examining the effects of undernutrition (Watkins et al., 2008) and in vitro culture (Watkins et al., 2007) during the preconceptual period in mice, we found increases in blood pressure in male offspring without noticeable changes in body weight during post-natal development. The lack of association between cardiac activity and body conformation has also been observed in another model of developmental programming in which male offspring of obese mice showed alterations during post-natal development in several variables associated with cardiovascular function independently of body weight (Blackmore et al., 2014).

It is believed that sex chromosome dosage differences between males and females are behind the gender-specific offspring phenotypes found on programming trajectories (Laguna-Barraza et al., 2012). In this scenario, gene dosage differences on sex chromosomes would affect differently the transcriptional activity of autosomal genes, resulting in sex-biased modulatory interactions in gene networks that would affect a large fraction of the genome, the ‘sexome’, which in turn will produce sex-specific phenotypes (Arnold and Lusis, 2012). Sex-specific differences in gene expression, mitochondrial function and epigenetic function are present at the preimplantation stage that could be expected to last beyond embryogenesis (Laguna-Barraza et al., 2012).

Interestingly, our AMA model showed several similarities with our previous study examining the long-term effects of extended in vitro embryo culture in mice (Watkins et al., 2007). In that study, a low cell number in both the trophectoderm and the inner cell mass was associated with increased body weight exclusively in females and high blood pressure in offspring regardless of sex (Watkins et al., 2007). However, even brief embryo culture (2 h) before blastocyst transfer was sufficient to minimally increase offspring blood pressure (Watkins et al., 2007). Hence, a combined effect of maternal aging of embryos and their in vitro culture and transfer most probably are behind the altered post-natal phenotype observed in the present study. Nevertheless, our aim was to develop a model that reflects to a certain extent the current use of ART in women of AMA (Cabry et al., 2014; Jackson et al., 2015). The use of ET in our study most probably accounts for the increase in body weight, which contrasts with previous reports of lower post-natal body weight in offspring of normally mated aged mice, coupled with the aged maternal tract environment (Tarin et al., 2005). However, notably, in both models the deviation in body weight after weaning was observed, not early, but later during post-natal development. Moreover, the decreased spleen weight associated with AMA we observed in our study was also found in mice offspring derived from natural pregnancies of old mothers (Albert et al., 1965). It is unknown at present if this decreased spleen weight will impact the functioning of the immune system.

Mitochondrial dysfunction associated with increased oxidative stress in oocytes has been suggested as a possible mechanism behind the long-term effects of aging (Tarin et al., 2003). Indeed, several studies in mice and humans have found altered mitochondrial function in oocytes from aged females (Kujo and Perez, 2012). Epigenetic modification has been also suggested as a potential mechanism underlying the periconceptual programming of disease occurrence in adulthood (Lucas, 2013) and several epigenetic modifications in oocytes from aged mice and humans have been documented (Ge et al., 2015). However, a direct association between these oocyte alterations and long-term programming of disease has not been reported. In addition, although experimental evidence has indicated that uterine aging is accompanied by molecular and morphological changes that can be detrimental for fertility (Nelson et al., 2013), the contribution of the microenvironment in the oviduct and uterus of aged mothers to long-term programming of phenotypes during post-natal life is largely unknown. Interestingly, a recent study with reciprocal ovarian transplants between young and old mothers indicated that the uterine environment, but not the oocyte, plays a crucial role in the development of congenital heart defects in offspring of old mice (Schulkey et al., 2015).

Given that the impaired oocyte quality and the development of congenital heart disease attributed to AMA can be ameliorated with nutritional or exercise interventions (Nehra et al., 2012; Schulkey et al., 2015), it is critical to determine if the risk of developing altered post-natal phenotypes associated with AMA can be decreased with similar interventions. We recognize that data from animal models cannot be extrapolated to humans, but they are valuable to develop conceptual
models that can produce verifiable hypotheses for eventual testing in the target species (i.e. humans).

In conclusion, in a murine ART – AMA model, we have shown that maternal aging can programme the development of impaired metabolic and cardiovascular function during post-natal life. Furthermore, our data also indicate that the preimplantation period is a developmental window in which adverse programming can be exerted by AMA and be independent of the maternal tract and systemic environment where pregnancy subsequently occurs. Hence, whilst human embryos display increased aneuploidy compared with mouse, we believe our data have implications for women of AMA undergoing assisted reproduction, including surrogacy programmes.

Acknowledgements

We are grateful for the technical support of the Biomedical Research Facility at the University of Southampton.

Authors’ roles

M.A.V. performed experiments, analysed data and wrote the manuscript. C.G.C.S. planned and performed experiments. N.R.S. performed experiments, and edited the paper. C.O. provided statistical expertise and edited the paper. T.P.F. conceived and designed the study, and edited the paper.

Funding

This work was supported through the European Union FP7-CP-FP EpiHealth programme (278418) to T.P.F. and the BBSRC (BB/F007450/1) to TPF. Funding to pay the Open Access publication charges for this article was provided by University of Southampton.

Conflict of interest

The authors have no conflicts of interest to declare.

References

Albert S, Wolf PL, O’Mara C, Barany W, Pryjma I. Influence of maternal age and parity on development of lymphoreticular organs of offspring in mice. J Gerontol 1965;20:530–535.

Albert BB, De Bock M, Derraik JG, Brennan CM, Biggs JB, Hofman PL, Cutfield WS. Increasing parental age at childbirth is associated with greater insulin sensitivity and more favorable metabolic profile in overweight adult male offspring. Am J Hum Biol 2015;27:380–386.

Arnold AP, Lusis AJ. Understanding the sexome: measuring and reporting sex differences in gene systems. Endocrinology 2012;153:2551–2555.

Blackmore HL, Niu Y, Fernandez-Twinn DS, Tarry-Adkins JL, Giussani DA, Ozanne SE. Maternal diet-induced obesity programs cardiovascular dysfunction in adult male mouse offspring independent of current body weight. Endocrinology 2014;155:3970–3980.

Cabric R, Merviel P, Hazout A, Belloc S, Dallecq A, Copin H, Benkhalfila M. Management of infertility in women over 40. Maturitas 2014;78:17–21.

Campbell A, Fishel S, Bowman N, Duffy S, Sedler M, Thornton S. Retrospective analysis of outcomes after IVF using an aneuploidy risk model derived from time-lapse imaging without PGS. Reprod Biomed Online 2013;27:140–146.

Carbone L, Chavez SL. Mammalian pre-implantation chromosomal instability: species comparison, evolutionary considerations, and pathological correlations. Syst Biol Reprod Med 2015;61:321–335.

Carwall CR, Steen LC, Joner G, Bulsara MK, Cinek O, Rosenbauer J, Ludvigsson J, Jane M, Svensson J, Goldacre MJ et al. Maternal age at birth and childhood type 1 diabetes: a pooled analysis of 30 observational studies. Diabetes 2010;59:486–494.

Constantinou C, Mpatsoulis D, Natsos A, Petropoulou PI, Zvintzou E, Traish AM, Voshol PJ, Karagannides I, Kypreos KE. The low density lipoprotein receptor modulates the effects of hypogonadism on diet-induced obesity and related metabolic perturbations. J Lipid Res 2014;55:1434–1447.

Crozier SR, Harvey NC, Inskip HM, Godfrey KM, Cooper C, Robinson SM; SWS Study Group. Maternal vitamin D status in pregnancy is associated with adiposity in the offspring: findings from the Southampton Women’s Survey. Am J Clin Nutr 2012;96:57–63.

Csermely G, Czeizel AE, Veszpremi B. Distribution of maternal age and birth order groups in cases with unclassified multiple congenital abnormalities according to the number of component abnormalities: a national population-based case-control study. Birth Defects Res A Clin Mol Teratol 2015;103:67–75.

Cutai D, Smajdor A. Postmenopausal motherhood reloaded: advanced age and in vitro derived gametes. Hypoxia 2015;30:386–402.

Donjacour A, Liu X, Lin W, Simbulan R, Rinaudo PF. In vitro fertilization affects growth and glucose metabolism in a sex-specific manner in an outbred mouse model. Biol Reprod 2014;90:80.

Dunson DB, Baird DD, Colombo B. Increased infertility with age in men and women. Obstet Gynecol 2004;103:51–56.

El Hajj N, Haaf T. Epigenetic disturbances in in vitro cultured gametes and embryos: implications for human assisted reproduction. Fertil Steril 2013;99:632–641.

Fall CH, Sachdev HS, Osmond C, Restrepo-Mendez MC, Victora C, Martorell R, Stein AD, Sinha S, Tandon N, Adair L et al. Association between maternal age at childbirth and child and adult outcomes in the offspring: a prospective study in five low-income and middle-income countries (COHORTS collaboration). Lancet Glob Health 2015;3:e366–e377.

Franasiak JM, Scott RT Jr. Embryonic aneuploidy: overcoming molecular genetics challenges improves outcomes and changes practice patterns. Trends Mol Med 2014;20:499–508.

Ge ZJ, Schatten H, Zhang CL, Sun QY. Oocyte ageing and epigenetics. Reproduction 2015;149:R103–R114.

Gillman MW, Rich-Edwards JW, Rifas-Shiman SL, Lieberman ES, Kleinman KP, Lipshultz SE. Maternal age and other predictors of newborn blood pressure. J Pediatr 2004;144:240–245.

Hardy K, Handyside AH, Winston RM. The human blastocyst: cell number, pathways and allocation during late preimplantation development in vitro. Development 1989;107:597–604.

Jackson S, Hong C, Wang ET, Alexander C, Gregory KD, Pisarska MD. Pregnancy outcomes in very advanced maternal age pregnancies: the impact of assisted reproductive technology. Fertil Steril 2015;103:76–80.

Jones KT, Lane SL. Molecular causes of aneuploidy in mammalian eggs. Development 2013;140:3719–3730.

Kenny LC, Lavender T, McNamme R, O’Neill SM, Mills T, Khashan AS. Advanced maternal age and adverse pregnancy outcome: evidence from a large contemporary cohort. PLoS One 2013;8:e56583.

Khali A, Syngelaki A, Maiz N, Zinevich Y, Nicolaides KH. Maternal age and adverse pregnancy outcome: a cohort study. Ultrasound Obstet Gynecol 2013;42:634–643.

Kleemann DO, Kelly JM, Rudiger SR, McMullen IC, Morrison JL, Zhang S, MacLaughlin SM, Smith DH, Grimson RJ, Jaensch KS et al. Effect of
periconceptional nutrition on the growth, behaviour and survival of the neonatal lamb. *Anim Reprod Sci* 2015;160:12–22.

Kujio LL, Perez GI. Ceramide and mitochondrial function in aging oocytes: juggling a new hypothesis and old players. *Reproduction* 2012;143:1–10.

Kwong WY, Wild AE, Roberts P, Willis AC, Fleming TP. Maternal undernutrition during the preimplantation period of rat development causes blastocyst abnormalities and programming of postnatal hypertension. *Development* 2000;127:4195–4202.

Lagarra-Barraza R, Bermejo-Alvarez P, Ramos-Ibeas P, de Fruutos C, Lopez-Cardona AP, Calle A, Fernandez-Gonzalez R, Perciessa E, Ramirez MA, Gutierrez-Adan A. Sex-specific embryonic origin of postnatal phenotypic variability. *Reprod Fert Dev* 2012;25:38–47.

Lammi N, Moltchanoa E, Blomstedt P, Eriksson JG, Taskinen O, Sarti C, Tuomilehto J, Karvonen M. The effect of birth order and parental age on the risk of type 1 and 2 diabetes among young adults. *Diabetologia* 2007;50:2433–2438.

Laopaiboon M, Lungbiganon P, Intarut N, Mori R, Ganchimeg T, Vogel JP, Souza JP, Gulmezoglu AM. Advanced maternal age and pregnancy outcomes: a multicountry assessment. BJOG 2014;121(Suppl 1):49–56.

Lawlor DA, Najman JM, Sterne J, Williams GM, Ebrahim S, Davey Smith G. Associations of parental, birth, and early life characteristics with systolic blood pressure at 5 years of age: findings from the Mater-University study of pregnancy and its outcomes. *Circulation* 2004;110:2417–2423.

Lee BK, McGrath JJ. Advancing parental age and autism: multifactorial pathways. *Trends Mol Med* 2015;21:118–125.

Lerch S, Brandwein C, Dormann C, Gass P, Chourbaji S. Mice age: does the age of the mother predict offspring behaviour? *Physiol Behav* 2015;147:157–162.

Lopes FL, Fortier AL, Darracanrearre N, Chan D, Arnold DR, Trasler JM. Reproductive and epigenetic outcomes associated with aging mouse oocytes. *Hum Mol Genet* 2009;18:2032–2044.

López-Cardona AP, Fernández-González R, Pérez-Crespo M, Alén F, de Fonseca FR, Orio L, Gutierrez-Adan A. Effects of synchronous and asynchronous embryo transfer on postnatal development, adult Health, and behavior in Mice. *Biol Reprod* 2015;93:85.

Lucas E. Epigenetic effects on the embryo as a result of periconceptional environment and assisted reproduction technology. *Reprod Biomed Online* 2013;27:477–485.

Master JS, Thouas GA, Harvey AJ, Sheedy JR, Hannon NJ, Gardner DK, Wlodek ME. Low female birth weight and advanced maternal age programme alterations in next-generation blastocyst development. *Reproduction* 2015;149:497–510.

Matthews JJ, Altman DG, Campbell MJ, Royston P. Analysis of serial measurements in medical research. *Br Med J* 1990;300:230–235.

Munné S. Chromosome abnormalities and their relationship to morphology and development of human embryos. *Reprod Biomed Online* 2006;12:234–253.

Myrskyla M, Fenelon A. Maternal age and offspring adult health: evidence from the health and retirement study. *Demography* 2012;49:1231–1257.

Myrskyla M, Elo IT, Kohler IV, Martikainen P. The association between advanced maternal and paternal ages and increased adult mortality is explained by early parental loss. *Soc Sci Med* 2014;119:215–223.

Nasr-Esfahani M, Johnson MH, Atkene RJ. The effect of iron and iron chelators on the in-vitro block to development of the mouse preimplantation embryo: BAT6 a new medium for improved culture of mouse embryos in vitro. *Hum Reprod* 1990;5:997–1003.

Nehra D, Le HD, Fallon EM, Carlson SJ, Woods D, White YA, Pan AH, Guo L, Rodig SJ, Tilly JL et al. Prolonging the female reproductive lifespan and improving egg quality with dietary omega-3 fatty acids. *Aging Cell* 2012;11:1046–1054.

Nelson SM, Telfer EE, Anderson RA. The ageing ovary and uterus: new biological insights. *Hum Reprod Update* 2013;19:67–83.

Rattanatravy L, MacLaughlin SM, Kleemann DO, Walker SK, Mulhaisler BS, McMullen IC. Impact of maternal periconceptional overnutrition on fat mass and expression of adipogenic and lipogenic genes in visceral and subcutaneous fat depots in the postnatal lamb. *Endocrinology* 2010;151:1195–1205.

Rehaj E, Paoloni-Giacobino A, Rimoldi SF, Fuster DG, Anderegg M, Somm E, Bouillet E, Alleman Y, Sartori C, Scherrer U. Mice generated by in vitro fertilization exhibit vascular dysfunction and shortened life span. *J Clin Invest* 2013;123:5052–5060.

Sauer MV. Reproduction at an advanced maternal age and maternal health. *Fertil Steril* 2015;103:1136–1143.

Savage T, Derraik JG, Miles HL, Mouat F, Hofman PL, Cutfield WS. Increasing maternal age is associated with taller stature and reduced abdominal fat in their children. *Proc Natl Acad Sci USA* 2007;104:19351–19356.

Schulkey CE, Regmi SD, Magana RA, Danzo MT, Luther H, Hutchinson AK, Panzer AA, Grady MM, Wilson DB, Jay PY. The maternal-age-associated risk of congenital heart disease is modifiable. *Nature* 2015;520:230–233.

Sinclair KD, Allgrucetti C, Singh R, Gardner DS, Sebastian S, Bispham J, Thurston A, Huntly JF, Rees WD, Maloney CA et al. DNA methylation, insulin resistance, and blood pressure in offspring determined by maternal periconceptional B vitamin and methionine status. *Proc Natl Acad Sci USA* 2007;104:19351–19356.

Smits LJ, Zielhuis GA, Jongbloet PH, Van Poppel FW. Mother’s age and daughter’s fecundity. An epidemiological analysis of late 19th to early 20th century family reconstitutions. *Int J Epidemiol* 2002;31:349–358.

Summers MC, McGinnis MK, Lawitts JA, Raffin M, Biggers JD. IVF of mouse ova in a simple optimized medium supplemented with amino acids. *Hum Reprod* 2000;15:1791–1801.

Sutcliffe AG, Barnes J, Belsky J, Gardner J, Melhuish E. The health and development of children born to older mothers in the United Kingdom: observational study using longitudinal cohort data. *Br Med J* 2012;345:e5116.

Tarin JJ, Vidal E, Perez-Hoyos S, Cano A, Balasch J. Delayed motherhood increases the probability of sons to be infertile. *J Assist Reprod Genet* 2001;18:650–654.

Tarin JJ, Gomez-Piquer V, Manzano C, Minarro J, Hermenegildo C, Cano A. Long-term effects of delayed motherhood in mice on postnatal development and behavioural traits of offspring. *Hum Reprod* 2003;18:1580–1587.

Tarin JJ, Gomez-Piquer V, Rauell F, Navarro S, Hermenegildo C, Cano A. Delayed motherhood decreases life expectancy of mouse offspring. *Biol Reprod* 2005;72:1336–1343.

Teare JE. Older maternal age and child behavioral and cognitive outcomes: a review of the literature. *Fertil Steril* 2015;103:1381–1391.

Verroken C, Kaufman J-M, Goemaere S, Toye K, Lapauw B. Maternal age at childbirth is associated with glucose metabolism in adult men. *Endocr Rev* 2015;36:FRI-616-FRI-616.

Wakefield SL, Lane M, Mitchell M. Impaired mitochondrial function in the preimplantation embryo perturbs fetal and placental development in the mouse. *Biol Reprod* 2011;84:572–580.

Watkins AJ, Platt D, Penabrock T, Wilkins A, Eckert JJ, Kwong WY, Osmond C, Hanson M, Fleming TP. Mouse embryo culture induces changes in postnatal phenotype including raised systolic blood pressure. *Proc Natl Acad Sci USA* 2007;104:5449–5454.

Watkins AJ, Ursell E, Panton R, Penabrock T, Hollis L, Cunningham C, Wilkins A, Perry VH, Sheth B, Kwong WY et al. Adaptive responses by mouse early embryos to maternal diet protect fetal growth but predispose to adult onset disease. *Biol Reprod* 2008;78:299–306.

Weidemann A, Lovas A, Rauch A, Andreas N, von Maltzahn J, Riemann M, Weih F. Classical and alternative NF-kB signaling cooperate in regulating adipocyte differentiation and function. *Int J Obes (Lond)* 2016;40:452–459.
Whincup PH, Cook DG, Shaper AG. Early influences on blood pressure: a study of children aged 5–7 years. Br Med J 1989;299:587–591.

Wilding M, Coppola G, De Icco F, Arenare L, Di Matteo L, Dale B. Maternal non-Mendelian inheritance of a reduced lifespan? A hypothesis. J Assist Reprod Genet 2014;31:637–643.

Williams CL, Teeling JL, Perry VH, Fleming TP. Mouse maternal systemic inflammation at the zygote stage causes blunted cytokine responsiveness in lipopolysaccharide-challenged adult offspring. BMC Biol 2011;9:49.

Wu LL, Dunning KR, Yang X, Russell DL, Lane M, Norman RJ, Robker RL. High-fat diet causes lipotoxicity responses in cumulus-oocyte complexes and decreased fertilization rates. Endocrinology 2010;151:5438–5445.

Yue MX, Fu XW, Zhou GB, Hou YP, Du M, Wang L, Zhu SE. Abnormal DNA methylation in oocytes could be associated with a decrease in reproductive potential in old mice. J Assist Reprod Genet 2012;29:643–650.

Yun Y, Holt JE, Lane SI, McLaughlin EA, Merriman JA, Jones KT. Reduced ability to recover from spindle disruption and loss of kinetochore spindle assembly checkpoint proteins in oocytes from aged mice. Cell Cycle 2014;13:1938–1947.