Effects of female dietary restriction in a rabbit growth line during rearing on reproductive performance and embryo quality

C. Naturil-Alfonso[1], R. Lavara[1,2], J.S. Vicente[1], F. Marco-Jiménez[1]

[1]Institute of Science and Animal Technology, Laboratorio de Biotecnología de la Reproducción, Universidad Politécnica de Valencia, Camino de Vera s/n, 46022 Valencia, Spain

[2]Department of Production and Animal Health, General Veterinary Health and the Science and Technology of Food Products, University CEU Cardenal Herrera, Tirant lo Blanc 7, 46115 Valencia, Spain

Abstract

Maternal diet prior to mating has an effect on reproductive performance. We analysed the effect of maternal dietary restriction during rearing on reproductive performance, the embryo development and foetal growth. Females were categorised in two groups; (i) does with *ad libitum* access to feed or (ii) restricted. Two experiments were performed: (1) After one month receptive females from both experimental groups were artificially inseminated and the reproductive performance was recorded during 3 reproductive cycles; at the first insemination, the body weight and perirenal fat thickness were recorded. (2) Females from both experimental groups were inseminated and 24 h later embryos were recovered and transferred to recipient females from a maternal line. Later, embryonic implantation was assessed at Day 14 by laparoscopy and foetal growth was monitored by ultrasound examination. In experiment 1, no differences in kindling rate was found, but prolificacy was showed to be higher in *ad libitum* does, which also were heavier than resticted ones. In experiment 2, no differences among does either in body weight, perirenal fat thickness or reproductive performance (ovulation rate and embryo recovery rate) were related to differences in feed intake. However, despite similar embryonic implantation losses, embryos from restricted females demonstrated higher foetal and gestational losses. Embryos from restricted does presented lower foetal growth than embryos from *ad libitum* does. Therefore, our results demonstrated that nutrition before first conception in a rabbit line selected for growth rate may impact on the embryo, and results in a disturbance in gestational losses and foetal growth over all reproductive life.

Introduction

Rabbit lines selected for growth rate are characterised by high feed intake, elevated growth rate and adult live weight (Estany et al. 1992), reduced reproductive performance (Mehaisen et al. 2004; Vicente et al. 2012), and also by an elevated disease incidence despite higher body condition score (Sánchez et al. 2012). Vicente et al. (2013a) reported that a rabbit paternal line selected for growth rate presented worse prenatal survival and gestational losses, of 50%, compared with maternal lines. Additionally, these and other authors (Ernst et al. 2000) showed that embryo genotype from females selected for growth rate had a significant effect on embryo survival at implantation and foetal survival rate. Hence this line could serve as a natural model to study the relationship between reproduction, energy balance and feed efficiency (Vicente et al. 2012).

On the one hand, survival and prenatal growth of mammalian embryos have shown to be influenced by the genotype of the embryo, genotype of the uterus providing the optimal embryo developmental environment and their interaction (Wilmut et al.1986). Developing embryos are influenced by maternal inherited effects, including cytoplasmic heritage, maternal age and maternal body size (Cowley 1991a,b). These maternal effects are often classified as epigenetic factors as they condition the progeny gene expression and therefore alter the relationship between genotype and phenotype in the progeny (Cowley et al. 1989; Atchley et al. 1991; Atchley and Hall 1991). Embryo genotype has been shown to modify uterine secretions, altering uterine tissue and affecting embryo survival rate and litter size (Wilson and Ford 1997). Additionally, Mocé et al. (2004) reported that the genotype of the embryos only has a relevant effect on embryo survival in a favourable maternal environment. Specifically, Vicente et al. (2013a) showed that embryo genotype in rabbit affects foetal survival and weight at day 25 of gestation,

while maternal genotype was responsible for implantation. More recently, it was shown that embryonic genotype influences prenatal survival and foetal weight at early gestation, and both embryonic and maternal genotypes influenced placental weight at middle and late gestation (Naturil-Alfonso et al. 2015).

On the other hand, reproductive function has shown to be highly related with reproductive performance (Fortun-Lamothe 2006). Although nutritional regimens after mating can have an effect, more data indicate that modifications to the nutritional regimen before mating may have greater impact on embryo survival and uniformity of litter size than the diet after mating (Ashworth et al. 1999a,b). Several reports have established that an altered nutritional regimen prior to mating can influence follicular/oocyte characteristics, but also that the maternal diet during pre-implantation development acts as a signalling input to the early embryo to regulate its future growth, as the peri-implantation period is a key period for future embryo development (Edwards and McMillen 2002; MacLaughlin et al. 2005; Watkins et al. 2008; Picone et al. 2011; Daoud et al. 2012). Moreover, maternal body weight or body condition before or immediately after conception may play a role in foeto-placental growth in early pregnancy, altering the weight of the foetal membranes and individual foetal weight (MacLaughlin et al. 2005).

The aim of this study was to evaluate the influence of feed restriction during rearing on reproductive performance and embryo development and survival, in a rabbit line selected for growth rate.

Material and Methods

All chemicals used in this study were purchased from Sigma-Aldrich Química S.A. (Madrid, Spain) unless stated otherwise.

Ethical Statement

The experiment was performed in accordance with the principles of animal care published by Spanish Royal Decree 53/2013 (BOE 2013). The animal studies were approved by the Committee of Ethics and animal Welfare of the Universidad Politécnica de Valencia (procedure 2015/vsc/PEA/00061). Researchers involved in the work with the animals possessed an animal experimentation license issued by the Spanish authorities.

Animals

Rabbits used in this experiment came from two commercial lines generated at the experimental farm of the Universidad Politécnica de Valencia. Animals were from a synthetic line selected for growth rate between weaning and slaughter time (9^{th} wk of life) for 36 generations (Estany et al. 1992; named line R). Recipient females were from a New Zealand White origin selected by family index for litter size at weaning for 43 generations (Estany et al. 1989; named line A). Does were housed individually at 12 wk of age, with free access to water, under 16 h light/8 h dark photoperiod, unless stated below.

At the age of four months, females from a line selected for growth rate were divided into two experimental groups: feeding *ad libitum* or restricted (provided daily with 130 g/day to accomplish with energy requirements for maintenance: 340 kJ day^{-1} kg^{-1} $LW^{0.75}$; Xiccato and Trocino 2010). Rabbits were fed with a commercial rabbit diet (on dry matter basis: 17.5% crude protein, 3.5% ether extract, 16.7% crude fibre, 2938 kcal/kg). After one month under these experimental conditions, females were reproduced. The feed intake of *ad libitum* does was determined by weighing the feeder at the beginning and end of each week, and all feed supplies given within a week were recorded. Additonally, when a restricted doe was tested as pregnant, the diet was

established as an *ad libitum* regimen.

Experiment 1. Effect of feeding regimen on reproductive performance

A total of twenty-six does (12 *ad libitum* and 14 restricted) were employed. Receptivity of does was determined observing the vulvar color and turgescency, considering receptive those with red/purple and swollen vulva. When receptive, does were artificially inseminated with 0.5mL of fresh heterospermic pool of males from the same line selected for motility criteria (more than 70% of motility rate and less than 15% of abnormal sperm) and diluted 1:5 with tris-citric-glucose diluent (Viudes-de-Castro and Vicente 1997). Immediately after insemination, ovulation was induced by an intramuscular injection of 1µg of Busereline Acetate (Suprefact, Hoechst Marion Roussel, S.A., Madrid, Spain).

Body weight and body composition of rabbit does were determined at the moment of the first AI. The perirenal fat thickness (PFT) of does was measured by ultrasound to evaluate body condition, as described by Pascual et al. (2000). Briefly, images were obtained with an ultrasound unit (Justvision 200 "SAS-320A" real-time sound machine, Toshiba), equipped with an electronic micro-convex transducer of multi-frequency (5.0, 6.0 and 7.0 –MHz; PVG-681S). PFT measures were indirectly obtained using the software of the ultrasound unit. At the moment of the PFT measurement, does were also weighted.

Fertility was evaluated during 3 reproductive cycles. Second and third inseminations were performed 12 days post-kindling, when insemination was tested as non-pregnant, next AI was performed 21 days after the previous one. Weaning was performed 30 d post-kindling. Fertility was defined at kindling as kindling followed insemination or no

kindling. The corresponding variable is the kindling rate (KR). Prolificacy was defined as number of total born/number of inseminations.

Experiment 2. Effect of feeding regimen on embryo development and foetal growth

Embryo recovery

From the previous females, nineteen donor does after weaning of third parity (7 *ad libitum* and 12 restricted) were artificially inseminated, as previously described. Twenty-four hours post-insemination, females were euthanised with an intravenous injection of 200 mg/kg of pentobarbital sodium (Dolethal, Vétoquinol, Madrid, Spain). Embryos were recovered by perfusion of each oviduct and uterine horn with 10 mL of pre-warmed Dulbecco Phosphate Buffered Saline supplemented with 0.2% of Bovine Serum Albumin. The ovulation rate and embryo recovery rate was recorded for each female. Ovulation and embryo recovery rate were calculated as the number of *corpora lutea* in both ovaries (OR) and the number of embryos recovered in each female (ER), respectively. Previously, females were weighed and after euthanasia, the perirenal fat was dissected and weighted.

Embryo transfer by laparoscopy

A total of 48 embryos from *ad libitum* females and 84 embryos from restricted females were transferred into oviducts by laparoscopy to 10 recipient does (11 to 16 embryos per recipient) following the procedure described by Besenfelder and Brem (1993). Ovulation was induced in recipient does with an intramuscular dose of 1 µg of Busereline Acetate 24 h before transfer. Briefly, recipients were sedated by intramuscular injection of 16 mg xylazine (Rompun; Bayer AG, Leverkusen, Germany). As surgical preparation for laparoscopy, anaesthesia was performed by intravenous injection of 16 to 20 mg of ketamine hydrochloride (Imalgene; Merial, S.A., Lyon, France) into the marginal ear vein. During laparoscopy, 12 mg of morphine

hydrochloride (Morfina; B. Braun, Barcelona, Spain) was administered intramuscularly. First, the abdominal region was shaved, and the animals were then placed on an operating table in a vertical position (head down at 45° angle). Only an endoscope trocar was inserted into the abdominal cavity. When the trocar was removed, the abdomen was insufflated with CO_2 and the endoscope was then inserted. For embryo transfer, embryos were aspirated in a 17-gauge epidural catheter (Vygon Corporate, Paterna, Valencia) introduced into the inguinal region with an epidural needle and then inserted into the oviduct through the infundibulum. After surgery, does were treated with antibiotics (0.1 mL/kg of procaine penicillin, Duphapen Strep; Pfizer, S.L.) and buprenorphine hydrochloride (0.08 mg every 12 hours for 3 days, Buprex; Esteve, Barcelona, Spain).

Embryonic implantation, delivery and losses rates

A total of 10 laparoscopies were carried out 12 d post-insemination. The number of implanted embryos (IE) and total born/litter size (TB) were recorded per female. Implantation and gestational losses were calculated, respectively, as the number of transferred embryos that did not reach the implantation or foetal stage. Foetal losses were defined as the proportion of implanted embryos that did not reach the foetal stage in pregnant does.

Ultrasound examination

Foetal growth was examined from day 12 post-insemination, at days 12, 14, 16, 19, 21, 23, 26 and 28 by a portable colour Doppler ultrasound device (Esaote, Spain) with 7.5 MHz linear probe (4-12 MHz range). Prior to examination, does were anaesthetised with ketamine 35 mg/kg and xylazine 5 mg/kg intramuscularly and the abdomen of the doe was clipped. Does were placed in a polystyrene cage where they were prevented from moving. The ultrasound examination was performed from right to left with the

probe in sagittal orientation and, after localisation of different foetal sacs, 5 to 7 foetal sac examinations per doe were performed. Foetus was measured from frozen frame pictures on the monitor, using the Esaote 16 ultrasound software (Fig. 1). Additionally, litter size was recorded at birth.

Foetal growth was determined by the linear distribution of crown-rump length (CRL) measurements, each 2 d of gestation (Vicente et al. 2013b).

A scheme of the experimental procedure is presented in Fig. 2.

Statistical analysis

Experiment 1. Effect of feeding regimen on reproductive performance

Kindling rate was analyzed by a GLM including the type of feeding with two levels (*ad libitum* or restricted) as a fixed factor. The error was designated as having a binomial distribution using the probit link function. Bionomial data for fertility was assigned a value of one if kindling followed insemination. Failure to insemination resulted in a score of zero. Prolificacy and body condition (weight and PFT) differences between groups were analyzed with a generalized linear model including the type of feeding with two levels (*ad libitum* and restricted) and parity (1 to 3) as a fixed effect.

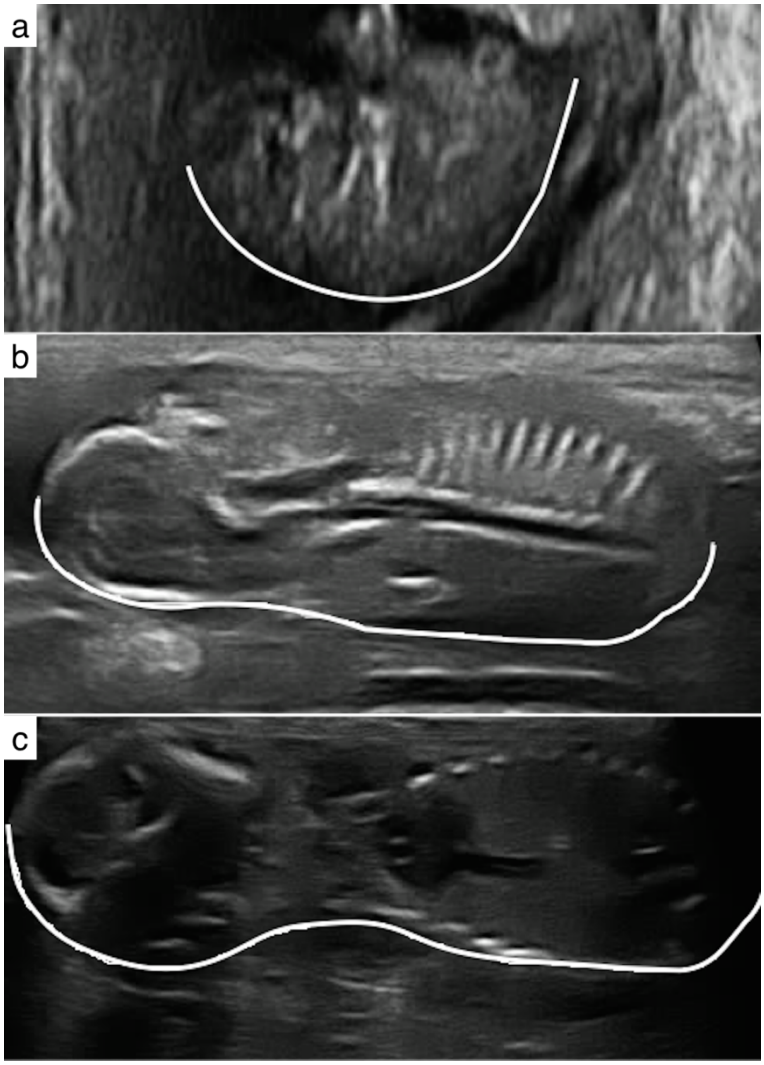

Figure 1. Ultrasound measurements of the crown-up length (CRL) of foetus at different days of gestation. (a) At 14 days (b) At 21 days (c) At 28 days.

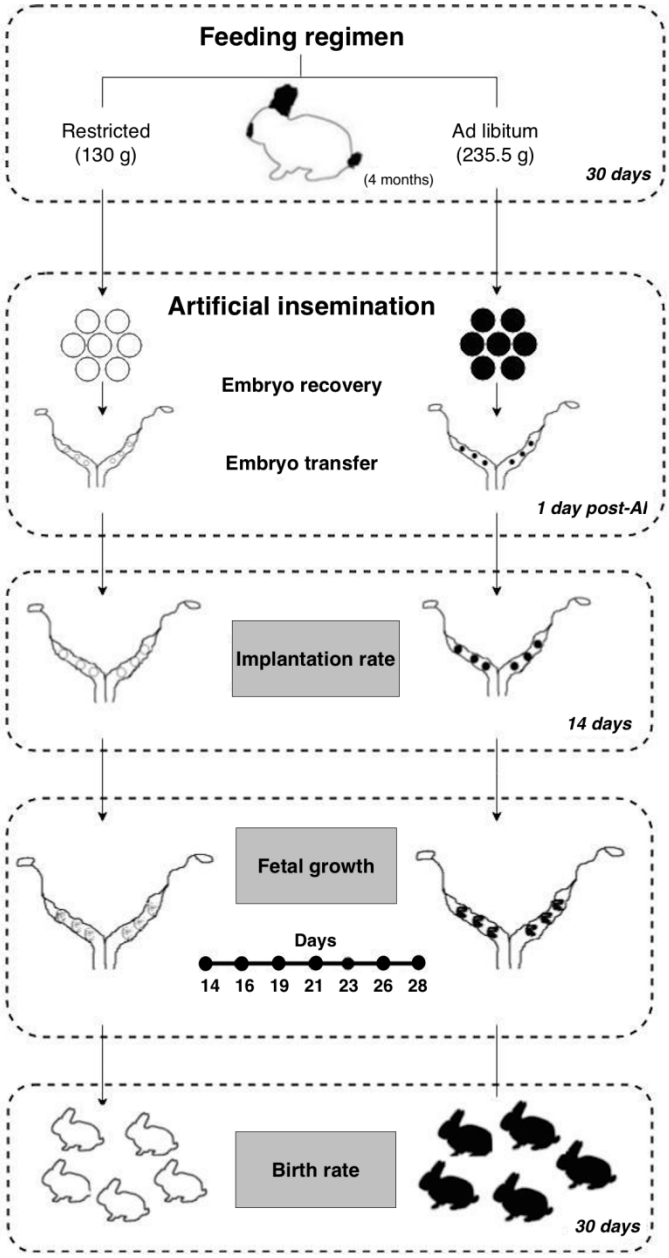

Figure 2. Schematic illustration of the experimental procedure used in experiment 2 to determine the effect of maternal feeding prior to mating on the embryo.

Experiment 2. Effect of feeding regimen on embryo development and foetal growth

For donor females, a GLM was performed to determine differences in body weight, PTF, OR and ER, among groups (*ad libitum* and restricted does).

To compare implantational, gestational and foetal losses between groups, a GLM was performed including the origin of embryo with two levels (*ad libitum* or restricted) as fixed effect. The error was designated as having a binomial distribution using the probit link function. Bionomial data for implantation, gestational and foetal losses per embryo were assigned a value of one if implantation occurred after transfer, if birth took place, or if birth took place from implantations, respectively. Failure to implant or continue development from implantation to birth resulted in a score of zero.

Litter size differences between groups were analysed with a general linear model including the origin of embryo with two levels (*ad libitum* or restricted) as fixed effect.

Regression Model Selection was used to determine a model that best evaluated foetal growth. The variables tested were female, litter size, and days of gestation. Additionally, the Group was included as a category variable (*ad libitum* or restricted). The model was selected by stepwise procedure based on Akaike Information Criteria (AIC). Later, a multiple regression analysis was performed to test the significance of each parameter.

Differences of $P < 0.05$ were considered significant. Data are shown as means ± standard error means (S.E.M.). All analyses were performed with SPSS 16.0 software package (SPSS Inc., Chicago, Illinois, USA, 2002).

Results

Experiment 1. Effect of feeding regimen on reproductive performance

During the three reproductive cycles, from the total of 26 females (12 *ad libitum* and 14 restricted), only 16 got pregnant after AI. Between groups, no differences in kindling rate were found (0.6 ± 0.08 *vs.* 0.4 ± 0.07, for *ad libitum* and restricted females,

respectively). Prolificacy was higher in *ad libitum* females during all of reproductive cycles (Table 1).

Donor females fed *ad libitum* showed significantly increased ingestion (235.5 ± 6.87 g/day) compared to the restricted group (130.0 g/day). At the moment of first AI *ad libitum* females showed to be heavier than restricted ones (5.3 ± 0.30 kg *vs.* 4.6 ± 0.28 kg, for *ad libitum* and restricted females, respectively), while no difference in PFT was detected (9.2 ± 0.30 mm *vs.* 8.7 ± 0.28 mm, for *ad libitum* and restricted females, respectively).

Table 1. Reproductive performance and body condition in *ad libitum* and restricted females after the rearing period.

Type	Feed consumption (g/day)	Kindling rate	Body weight (kg)	PTF (mm)
AD (N= 12)	235.5 ± 6.87	0.6 ± 0.08	5.3 ± 0.30[a]	9.2 ± 0.30
R (N = 14)	130.0 ± 0.00	0.4 ± 0.07	4.6 ± 0.28[b]	8.7 ± 0.28

	Kindling order			
	1st	2nd	3rd	Total
AD (N= 12)	4.1 ± 0.30[a] (N=12)	5.0 ± 0.32[a] (N=11)	5.3 ± 0.56[a] (N=8)	4.8 ± 0.17[a]
R (N = 14)	3.2 ± 0.28[b] (N=14)	3.1 ± 0.27[b] (N=14)	3.2 ± 0.24[b] (N=11)	3.2 ± 0.15[b]

AD, female fed *ad libitum* during rearing; R, female fed restricted during rearing; N, number of females. Values with different superscripts in the same column are statistically different ($P < 0.05$).

Experiment 2. Effect of feeding regimen on embryo development and foetal growth

Donor females

No significant differences were found between females fed *ad libitum* and restricted either in body weight or in perirenal fat weight (6.9 ± 0.23 and 6.5 ± 0.17 kg, body weight and 142.3 ± 19.46 and 103.06 ± 14.87 g fat weight, for *ad libitum* and restricted females, respectively).

Likewise, reproductive parameters showed no significant differences between both types of females. From the nineteen females, five of them (3 *ad libitum* and 2 restricted) did not produce embryos. From the others 14, ovulation rate was 13.3 ± 1.65 and 12.9 ± 1.08 for *ad libitum* and restricted donor females, respectively. The embryo recovery frequency was 92% and 95%, for *ad libitum* and restricted donor females respectively, while the embryo recovery was 12.3 ± 1.56 and 12.3 ± 1.02, respectively.

Embryonic implantation, birth rate, foetal and gestational losses

Figure 3 shows implantation, gestational and foetal losses of *ad libitum* and restricted transferred embryos. Whereas implantation losses did not show differences between both groups of embryos (0.20 ± 0.044 *vs.* 0.25 ± 0.063, for restricted and *ad libitum* derived embryos, respectively), gestational and foetal losses showed significant differences between them (0.58 ± 0.054 *vs.* 0.40 ± 0.071 and 0.48 ± 0.061 *vs.* 0.19 ± 0.066, for restricted and *ad libitum* derived embryos, in gestational and foetal losses, respectively). Additionally, LS was higher for *ad libitum* transferred embryos (9.3 ± 0.09 *vs*. 7.5 ± 0.07, total born for *ad libitum* and restricted embryos, respectively).

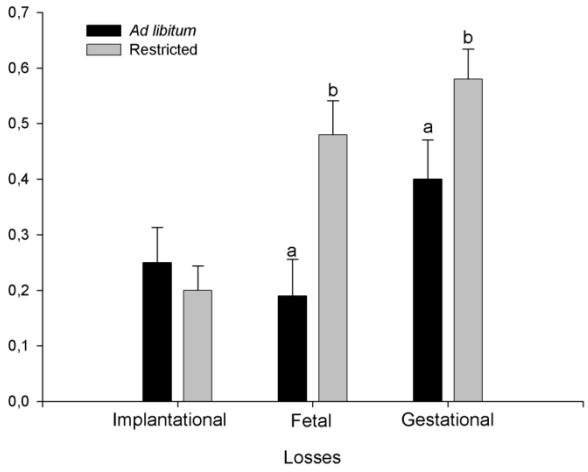

Figure 3. Implantational, foetal and gestational losses for embryos derived from restricted and *ad libitum* fed females. Bars with different letters are significantly different ($p < 0.05$).

Foetal growth

The regression model that includes Days of gestation (D) and Group (G: Ad libitum or restricted) as independent variables could explain nearly 95% of the variation in foetal growth measured as CRL (residual standard error: 0.67). The standardized coefficient was β=0.49 (S.E: 0.15, $p<0.05$) for days of gestation, and β=-0.19, (S.E: 0.01, $p<0.05$) for group (referred to restricted group). These results showed differences in growth rates measured in CRL between both groups with the *ad libitum* embryos showing higher foetal growth (+0.19 cm/day) than restricted embryos at the same day of gestation.

Discussion

It is widely known that maternal nutrition and body condition prior to mating or immediately after, during pre-implantation development, affects the establishment and the growth trajectory of the foetus (MacLaughlin et al. 2005; Watkins et al. 2008; Picone et al. 2011; Daoud et al. 2012). The current study shows that does from a line selected by growth rate with feed restriction during rearing, might be disadvantaged in terms of oocyte reserve quality or fertilization process that conditioned embryo survival, increasing foetal and gestational losses and limiting foetal growth.

The experimental group developed and analyzed in experiment 1 showed that *ad libitum* females presented higher fertility than restricted ones, although not statistically significant, which could be explained by the relative low number of animals. Indeed, *ad libitum* are heavier and present higher prolificacy than restricted ones. The feeding regimen leading to a higher body weight and prolificacy could be altering the fertilization process, uterine environment or the oocyte, which would be manifested at the embryo level. With this aim, in the experiment 2 we analyzed the effect of the feeding regimen on the embryos produced by both types of females.

First, feed intake for does that were fed *ad libitum* was evaluated. In general, heavy does are heavier as a consequence of higher feed intake or a higher growth potential (Rommers et al. 2002), which was not the case in our study. Our results showed that although *ad libitum* diet showed an ingestion increase of 105.5 g/day more than restricted, *ad libitum* does were not heavier and over-fattening had not occur at the moment of embryo recovery. Picone et al. (2011) reported similar results in rabbits, finding no differences in body weight gain and in fat weight between females following a hypercholesterolemic and hyperlipidic diet and females following a control diet from week 10 to 18 of age. Additionally, Rommers et al. (2002) indicated that after rearing period, heavy does had not formed relatively more fat tissue compared to medium and small does. Moreover, as other authors have reported in females submitted to alter feeding regimens, our data did not reveal differences in ovulation and embryo recovery rate and embryo recovery percentage between *ad libitum* and restricted donor females (Kwong et al. 2000; Tripp et al. 2000; Cardinali et al. 2008). However, nutritional diet during rearing not only affects body condition, but also has shown to alter metabolic and reproductive hormones influencing fertility (Brecchia et al. 2006).

In our work, embryos were transferred to females from a maternal line to analyse only the embryonic origin effect and discard the endometrial environment effect, as this maternal environment has been shown to be better for embryo development and foetal survival than the uterine environment of line R (Naturil-Alfonso et al. 2015). When embryos from *ad libitum* and restricted does were transferred to a female from a maternal rabbit line, they reached a similar implantation rate, and therefore, similar implantation losses were observed. However, foetal and gestational losses were higher in embryos originated in restricted females, doubling gestational losses of *ad libitum* ones. In a similar way, litter size of *ad libitum* embryos was higher than in restricted.

Discrepancies are found in the literature about the influence of diet and body condition on pregnancy establishment and miscarriages. On the one hand, similar implantation rate and litter size in females following a control or an *ad libitum* low protein diet has previously been reported (Kwong et al. 2000). On the other hand, decreased pregnancy rates and high miscarriages rates have been observed in response to obesity in women (Robker 2008) or in ewes with high feed intake (Parr et al. 1987).

However, our results are in contrast to those reported in rabbit. Petrere et al. (1993) described no effect of feed restriction (150 g/day) on post-implantation losses compared with *ad libitum* ones (220 g), similar to Cappon et al. (2005) in foetal viability. This disagreement may be produced by the line studied, as these studies worked with a maternal line while our work was carried out with a paternal one. Rabbit paternal lines are characterised by reduced reproductive performance, with the highest implantational, gestational, foetal and perinatal losses (Mehaisen et al. 2004; Vicente et al. 2012). Previously, it has been shown that embryonic genotype influences implantation and foetal survival, being lower in does of paternal lines when compared with a maternal line as reference, but maternal genotype also affects implantation rate (Vicente et al. 2013a). More recently, Naturil-Alfonso et al. (2015) reported that embryonic genotype influences prenatal survival at early gestation, which is in contrast with our results as the survival rate at Day 14 (implantation rate) was the same between groups. Thus, the differences between groups may be attributed to the model employed.

In addition, restricted derived foetuses showed lower foetal growth from implantation to birth (Fig. 4). The vast majority of the studies analysing foetal growth in feed regimen altered conditions reported alterations in placental length, foetus birth weight and post-natal growth (Kwong et al. 2000; MacLaughlin et al. 2005; Picone et al. 2011; Cordier et al. 2013). As in our results, it was previously shown in rabbit does that

hypercholesterolemic and hyperlipidic diet led to foetal growth differences between groups, with the main differences found at term of gestation (days 27 and 28) (Picone et al. 2011; Cordier et al. 2013).

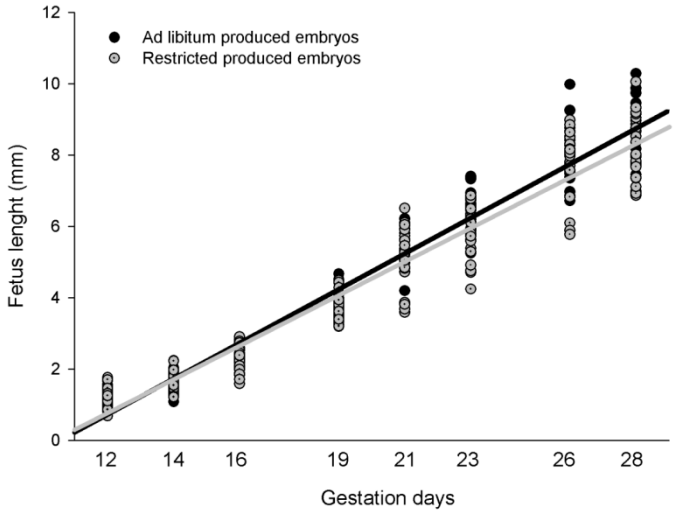

Figure 4. Regression lines of foetal growth from day 14 to 28 of gestation in surrogate females for *ad libitum* and restricted produced embryos.

Our findings confirm that foetal growth is altered by the feeding regimen of the mother during rearing, and further suggest that this developmental trajectory is established during oocyte development and maturation. Previous studies reported that effects of under- or over-nutrition on embryo development begin at the ovarian levels (Wakefield et al. 2008; Igosheva et al. 2010; Luzzo et al. 2012). During oocyte growth phase, RNA and protein synthesis and storage take place (Crozet et al. 1981; Godsen et al. 1997). Meiotic progression up to MII, fertilisation and early embryonic development must be maintained by the stored RNA and proteins (Bachvarova 1992). Additionally, acquisition of DNA methylation is mostly performed in antral follicles and MII-ovulated oocytes (Saitou et al. 2012). This DNA methylation or methylation errors showed to still be present at blastocyst stage (Denomme and Mann 2012). Deficiencies in these processes could lead to a decrease in developmental competence (Anguita et al.

2008). In fact, it was shown that pre-implantation embryo development can be reprogrammed during the final stage of oocyte maturation (Leroy et al. 2015). Recently, Wu et al. (2015) have reported that oocytes from obese female mice fertilised *in vitro* have reduced potential to form blastocysts *in vitro*, but also when they were transferred to surrogate females foetal growth was higher in foetuses derived from oocyte of obese mice than from controls. These authors indicated that maternal obesity before conception alters developmental growth trajectory of the foetus already established during oocyte development and maturation, within the follicle or before fertilisation. This is consistent with our work, where results evidence those differences in nutrition before conception may affect oocytes in some way that is subsequently inherited by the embryo and is manifested at the level of foetal and gestational losses and foetal growth. In contrast, acute fasting of 72h prior to AI has shown to alter metabolic and endocrine markers, but did not affect follicle and oocyte development in rabbits (García-García et al. 2011). However, our study focuses in long-term consequences (3 reproductive cycles and posterior embryos) of a long-restricted period during rearing, while García-García et al. (2011) studied the direct consequences of short fasting period on nulliparous does, which did not involved follicular development and oocyte competence as ovulated follicles of this cycle were already in development during the fasting period.

The alterations observed in this line selected by growth rate may be due to feed restriction. It could be that a slight negative energy balance during rearing in the restricted does might have affected the oocyte. Specifically in rabbits, Arias-Álvarez et al. (2009) reported that metabolic status of the female is associated with reproductive outcomes, as this status determines the acquisition of oocyte developmental competence, in terms of nuclear and cytoplasmic maturation.

As stated previously, these lines have serious reproductive issues, with high foetal and perinatal losses (Vicente et al. 2012). The way in which an animal accommodates a nutritional change will depend upon its priorities (Friggens 2003). The genetic characteristics of this line could make does prioritise their own maintenance to that of the offspring, as there is a genetic component controlling size and mobilisation of body fatness, with high priority in safeguarding body reserves of the animals (Theilgaard 2006). Thus, if these females are restricted during rearing they may have a lack of dietary intake capacity to meet the demands. So, when they eat *ad libitum* they have enough energy to supply their needs and also for the offspring, and consequently the gestational losses are reduced and the foetal growth is higher.

Therefore, we showed that a restricted diet in females selected for growth rate during rearing may alter the oocyte in some undetermined way, and consequently the embryo, which leads to an increase in the foetal and gestational losses when compared with losses of embryos from females with an *ad libitum* diet, and increases foetal growth and litter size. Considering this, and that (i) the last study reporting implantational, foetal and gestational losses for the rabbit line employed in this work (line R) showed results similar to ours in restricted embryos for foetal and gestational losses (Vicente et al., 2012); and (ii) embryonic genotype is mainly responsible for these losses; we can affirm that with a restricted diet during rearing we have altered the oocyte and embryo, and so increased the foetal and gestational losses and decreased foetal growth.

In conclusion, although no differences in weight, perirenal fat thickness, ovulation and embryo recovery rates are found between females fed *ad libitum* or restricted during rearing, foetal and gestational losses and foetal growth are altered between embryos produced by these females. Our study shows that when embryos came from feed restricted females, the gestational losses are those expected for this line and foetal

growth is lower, while with an *ad libitum* diet gestational losses are reduced by half. It may be that restrictive nutrition affects oocytes and so alters the embryo, which in turns reduces gestational losses and foetal growth. Our results highlight the importance of periconceptional diet in oocytes and embryos of a rabbit line selected by growth characteristics. Thus, they call for further studies on oocytes and embryos to better understand the mechanisms responsible for the effects observed in this rabbit model, and also on the metabolism of this line to elucidate their energy management during gestation.

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Conflict of interest

None of the authors have any conflict of interest to declare.

Author contributions

C. Naturil-Alfonso, J.S. Vicente and F. Marco-Jiménez have designed, contributed to the acquisition, analysis and interpretation of data and drafted and revised critically the manuscript. R. Lavara has contributed to the acquisition and interpretation of data and revised critically the paper.

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