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HISTROTROPHIC NUTRITION AND EARLY EMBRYO DEVELOPMENT

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ABSTRACT

Histrotrophic nutrition was manipulated in adult Merino ewes during the course of a single reproductive cycle, from 18 days before until 6 days after ovulation. The effect of this relatively short-term exposure to high feed intake (H: 1.5 x maintenance ration), maintenance intake (M: 1x maintenance ration) or low feed intake (L: 0.5 x maintenance) was examined on embryo development, composition of oviductal fluid and pregnancy outcome in the ewes that either ovulated naturally or were induced to superovulate. Live weight was significantly ($P<0.05$) reduced in sheep on L- compared with a M- or H- feed intake regime. The mean number of embryos collected, the mean number of corpora lutea (CL), and the proportion of embryos/CL recovered, was not influenced significantly by differences in feed intake. However, embryos from sheep with an L feed intake during the peri-conceptional period consistently contained a significantly ($P<0.05$) increased number of cells, resulting from a specific increase in the number of trophectoderm (TE) cells. Manipulating feed intake across the pre- vs. post-ovulatory periods revealed that exposure to a Low feed intake in the 6-days post-ovulatory period caused this increase in the number of TE cells.

Overall, these studies indicate that short-term changes in feed intake can significantly alter embryo development with potential consequences for subsequent fetal development. The way in which these short-term changes influence development has not been resolved, but a number of exciting avenues are presented for further investigation. The findings are relevant to both human and animal and to improving our understanding of basic developmental process in reproduction.

Keywords: embryo; nutrition; inner cell mass; trophectoderm; sheep

INTRODUCTION

Nutrition has both long-term and short-term effects on various parameters of livestock reproduction (O'Callaghan and Boland, 1999). Dietary intake can affect plasma concentrations of steroids such as progesterone and also intra-follicular concentrations of factors such as IGF-1 and IGF-2 (Houseknecht *et al.*, 1988). High nutrition has a negative effect on oocyte quality (cumulus cover and cytoplasm granulation), with animals on *ad libitum* high-energy diets particularly at risk (O'Callaghan *et al.*, 2000). Furthermore, several studies have indicated that excessive feeding has been associated with a reduction in the yield and quality of embryos in cattle (Blanchard *et al.*, 1990; Mantovani, *et al.*, 1993; Nolan, *et al.*, 1998; Boland and Callaghan, 1999; Butler, 2000) and sheep (McEvoy *et al.*, 1995a). It has been suggested that these results are due to changes primarily at the level of the follicle or oocyte (Dunne *et al.*, 1997; Boland *et al.*, 2001). Deleterious effects on embryo development of excessive nutrition around mating are also evident following superovulation (Mantovani *et al., 1993;* Yaakub *et al.*, 1999; Nolan *et al.*, 1998).

Mantovani *et al.* (1993) reported that superovulatory responses in cattle were lower and the yield of good quality embryos reduced in heifers that had been exposed to high levels of nutritional concentrates in association with reduced roughage. Despite substantial progress being made in understanding factors that control follicle growth (Adams, 1994) and ovulation (Gong et al. 1993), the mechanism by which nutrition influences embryo quality is not clear. Maurasse *et al.* (1985) reported that high feed intake in heifers can influence ovarian function by reducing the number of large non-atretic follicles and increasing the number of small non-atretic follicles. Assey *et al.* (1994) reported that impaired developmental capacity of superovulated oocytes was associated with a number of subcellular abnormalities (structural changes in the degree of detachment of interchromatin-like granules within the oocyte nucleolus) compared with oocytes from untreated cows. Nutritional excess may exacerbate these deviations.

While the effects of an elevated plane of nutrition on embryo quality are evident in superovulated ewes maintained on improved diets for extended time periods (up to 6 months) (Maurasse et al. 1985; Sutton et al. 1986; Dunn and Moss, 1992; Yaakub et al. 1997; O'Callaghan and Boland, 1999; Boland et al. 2001), less is known of the effects of short-term changes in diet. Ovulation is increased in sheep in response to an increased dietary intake for a relatively short time (Coop 1966a and b). There is little information however, regarding the effects of the level of nutrition during the cycle of conception on embryo development. The primary objective of this study was thus to examine the effect of changes in nutrition (from 18 d before until 6 d after conception) on embryo production and quality in the superovulated ewe. The results of this study indicate that level of nutrition significantly influences several parameters including blastocyst cell number and cell lineage differentiation. We subsequently determined if these changes resulted from histotrophic nutritional influences during pre-ovulatory or post-ovulatory periods. A preliminary account of this work has been published (Kakar *et al.* 2001).

MATERIALS AND METHODS

Animals

All procedures in this study were conducted according to guidelines of the Australian Code of Practice for the Use of Animals for Scientific Purposes and received approval from the Adelaide University Animal Ethics Committee and the South Australian Research and Development Institute Animal Ethics Committee. South Australian Merino ewes, managed under normal husbandry practices, were moved prior to the experimental periods to covered yards for 10-15 days and fed pelleted sheep rations of standard composition (Table 1) at a rate of 600 g/day/ewe. During the experiments, ewes were housed indoors in single pens exposed to natural photoperiod. Each pen had an individual feed bin and water was provided *ad libitum*.

All ewes (4-5 years old of 55-65 kg) were weighed at the beginning and end of each experiment. Daily energy need for maintenance was calculated from the Agricultural Research Council (1980) feeding standards using the Rumnut computer program (Pullman & Hughes, 1986). A weighed quantity of feed was offered daily at 09:00 h and any feed residues were collected every 2-3 days and weighed to determine feed intake.

Experimental design

Experiment 1 aimed to examine the effect of short-term (one cycle) changes in nutrition on blastocyst

development and quality. This experiment was designed as a randomised complete block design with 3 blocks of 16 sheep of comparable body weight and condition. The three dietary treatments were high feeding (1.5 x daily energy needs for maintenance), moderate feeding (1.0 x daily energy needs for maintenance) and low feeding (0.5 x daily energy needs for maintenance). These treatments are identified by the H, M and L notations respectively. All 48 ewes were kept for at least two weeks on maintenance ration before the commencement of the trial. The nutritional treatments were imposed from 18 d before until 6 d after the expected time of ovulation.

Experiment 2 aimed to discriminate between the changes to the embryo resulting from nutritional influences during the preovulatory and postovulatory periods. In this experiment, 76 sheep were randomly allocated to 12 randomised blocks on the basis of their initial weight. Within each block, between 6 and 8 sheep were randomly assigned to one of six nutrition regimes. These regimes consisted of a combination of the dietary treatments used in Experiment 1 but were fed for three different time periods, as shown in Table 1. For example, sheep assigned to treatment 1 were fed a diet with a high nutritional value for the course of the experiment (HHH) and sheep assigned to treatment 6 were fed a low nutritional diet for the course of the experiment (LLL). In comparison, sheep assigned to treatment 2 to 5 were fed the M diet (control), for the first 6 days of the experiment (Day –12 to –6 before the expected time of ovulation), before being switched to a H or L diet at Day –6 and again after ovulation (Day 0) until embryos were collection 6 days later (+6 days).

Superovulation

Superovulation was induced in ewes using a progestagen pessary (45 mg flugestone acetate; Intervet, Paris, France) for 12 days followed by treatment with follicle stimulating hormone (FSH; Ovagen, ICP, Auckland, New Zealand). The latter treatment consisted of six injections given twice daily, commencing 48 h before pessary removal and equivalent to 7.9mg NIADDK- oFSH-17 standard. Pregnant mare serum gonadotrophin (Pregnecol, 500 iu i/m; Horizon, New South Wales, Australia) was administered at the time of the first FSH injection. Synthetic gonadotrophin releasing hormone (Fertagyl, 50 μg per ewe; Intervet) was administered (i.m) 27 h after pessary removal.

Artificial insemination and embryo collection

Fresh semen was collected using an artificial vagina and diluted (1:4) with phosphate buffered saline + 10% sheep serum. Ewes were inseminated by laparoscopy with approximately 20 x 10^6 spermatozoa per uterine horn placed directly into the lumen. Insemination occurred approximately 40 h after pessary removal. Semen was provided from the same two rams of proven fertility in each experiment.

Six days after insemination, embryos were collected under general anaesthesia by mid-ventral laparotomy. The number of corpora lutea were recorded and each uterine horn was flushed with 20 ml phosphate-buffered saline containing 5% heat inactivated sheep serum. Embryos were recovered from the flushing medium within 5 min of collection using a stereomicroscope.

Differential staining

Blastocyst were processed immediately where as morulae and compact morulae were held in culture medium (SOF + BSA + amino acids) overnight in a humidified atmosphere of 5% CO_2: 5% O_2: 90% N_2 at 38.5 ^{0}C before processing. The nuclei of the trophectoderm (TE) and inner cell mass (ICM) cells of the

embryos were differentially labelled with polynucleotide-specific fluorochromes using a slight modification (Van Soom *et al.* 1996) of the method of Hardy *et al.* (1989). Briefly, the partial digestion of the zona pellucida was achieved by exposing the embryos, in groups of three to six, to prewarmed (37^{0}C) pronase solution (0.5% Protease; Sigma Chemical Co., St. Louis, MO) until the zona became wrinkly (after about 1 min to 1½ min). Thereafter, the zona was completely removed sometimes with mechanical assistance using a narrow bored pipette. The zona-free blastocysts were washed in medium containing 10% v/v sheep serum in PBS (Ca^{2+} and Mg^{2+} free) containing 4% w/v polyvinylpyrrolidone (PVP; ICN Biochemicals, Cleveland, Ohio 44128). Blastocysts were then incubated in 10mM trinitrobenzenesulfonic acid (TNBS; Sigma) in PBS containing 4 mg/ml PVP (pH 7.4) on ice for 10 min. TNBS combines with N-terminal amines (Voet and Voet, 1990) and thus labels certain phospholipids or proteins in the blastomere membrane with covalently bound trinitrophenol groups. The embryos were washed three times in PBS$^-$/PVA and incubated in 0.1 mg/ml anti-DNP-BSA (secific antibody activity 1.3 mg/ml; Sigma) at 37^{0}C for 10 min. The anti-DNP-BSA is a commercially available antiserum raised in rabbits against dinitrophenol groups (DNP), which also cross reacts with trinitrophenol groups. Excess antiserum was then washed away in PBS$^-$/PVA before exposure to complement, resulting in selective antibody-mediated complement lysis of the TE. After a quick wash, the embryos were finally incubated in a 1:10 dilution of guinea pig complement (IMVS; Adelaide, Australia) in PBS$^-$, which included 10 μg/ml propidium iodide (PI; Sigma) for 15-30 min at 37^{0}C.

After the complement mediated cell lysis and the staining of the lysed cells by propidium iodide, the embryos were briefly washed in PBS to remove complement proteins, before being fixed with ice-cold absolute ethanol. After 5 min of fixation, the embryos were transferred into the second fluorochrome 10 μg/ml in PBS$^-$ bisbenzimide (Hoechst 33342; Sigma) in absolute ethanol at room temperature or at 4^{0}C overnight, which penetrates both lysed and intact cell membranes and therefore stains all the nuclei of the embryo. After incubation, the embryos were washed briefly in PBS$^-$/PVA and then transferred to a drop of glycerol containing Hoechst 33342 (1mg/ml) on a microscope slide and gently covered with a coverslip supported at each corner with vaseline: paraffin wax mixture (9:1). The embryos were carefully squashed and disaggregated using a pen and then again examined in whole mount using a fluorescent microscope (Olympus BH2; Osaka, Japan). The number of ICM (blue) and TE nuclei (pink to red) were counted directly under the microscope. Photomicrographs were taken on Fuji colour positive ASA 400 film corrected to ASA 800 for dark ground using an Olympus BX 60 fluorescent microscope.

Statistical analysis

Analyses of the two experiments were performed in GenStat for Windows, 5th Edition (Harding et al. 2000), using the procedure Restricted Maximum Likelihood (REML) (Patterson & Thompson, 1971). REML is similar to analysis of variance (ANOVA) in that it accounts for more that one source of variation. However, REML has the added advantage of being able to handle grossly unbalanced data. In a REML analysis, the treatment effects were tested using a Wald test at the 5% level. This test statistic is approximately Chi-squared distributed with the appropriate degrees of freedom. The assumptions of REML analysis are that the data is normally distributed with constant variance. The validity of these assumptions is examined using various diagnostic plots, such as the residuals versus fitted values plot. These plots were examined for each response and there was no indication that these assumptions were violated.

The null hypothesis is that there were no significant differences between the dietary treatments versus the alternative that at least two dietary treatments were significantly different. However, for Experiment 2 this

treatment allocation would not detect whether there was a significant difference between the High and Low nutritional diets before compared with after mating. Therefore an alternative treatment structure was considered so that the interaction between the pre-mating (-6 Days) diet allocation (H or L) and post-mating (after A.I) diet allocation (H or L) could be detected. Hence a nested model was considered, in which the factorial structure for pre-mating (with two levels, L and H) and post-mating (also with 2 levels) occurs within a factor treatment that distinguishes between the controls (either LLL or HHH) and the modified diets.

RESULTS

Experiment 1

Mean liveweight was significantly ($p<0.05$) lower in the L group than in other groups at the end of the study period (Table 3). All groups lost weight during this period although the loss was not significantly different between the H and M treatments. The mean number of corpora lutea and the proportion of embryos collected of corpora lutea were not significantly influenced by diet. Of those embryos collected, not all had reached blastocyst and so an analysis was performed on the proportion of embryos that did not reach blastocyst. There were no statistically significant differences between diets for this response ($p=0.1$). However embryos from sheep fed L had significantly ($P< 0.05$) more cells (74.68 ± 1.45) compared with those sheep fed H (62.01 ± 0.84). The TE:ICM ratio for sheep fed the L ration (0.73 ± 0.004) was significantly ($P < 0.05$) higher than for the H (0.69 ± 0.003) or M (0.70 ± 0.004).

Experiment 2

Body weight was significantly ($P<0.05$) reduced and weight loss was significantly greater ($P<0.05$) in those sheep fed the L ration regardless of whether this treatment occurred before or after mating. The number of corpora lutea and the proportion of embryos collected of corpora lutea were not significantly influenced by diet (Table 5). Significantly ($P<0.01$) fewer embryos had developed to the blastocyst stage in sheep fed the H diet after mating compared with the L diet (Table 4). The total number of cells per embryo was significantly ($P<0.05$) reduced for sheep receiving the H ration for the whole trial period or after mating compared with other rations. However, this figure did not differ significantly when treatments were imposed before mating ($P=0.488$). The TE:ICM ratio differed significantly ($P<0.001$) between treatments only when treatments were applied after ovulation, with L sheep having more TE cells than H sheep.

Table 1. The design for experiment 2 indicating the periods of nutritional treatment relative to the day of ovulation (day 0). Ewes were fed either a high (H, 1.5 x maintenance), medium (M, 1 x maintenance) or low (L, 0.5 x maintenance) diet.

Regime	-12 to -6 Days	-6 to 0 Days	0 to +6 Day
1	H	H	H
2	M	H	H
3	M	H	L
4	M	L	H
5	M	L	L
6	L	L	L

Table 2. Composition of feed used in Experiments 1 and 2

Test	*Method*	*Units*	
Moisture	NIR	%	9.9
Dry matter	NIR	%	90.1
Ash	NIR	%	8.5
Crude protein	NIR	% of dry matter	19.1
Acid detergent fibre	NIR	% of dry matter	23.9
Neutral detergent fibre	NIR	% of dry matter	33.3
Digestibility	NIR	% digestible DM	69.3
Fat (ether extract)	Wet	% of dry matter	1.6
Metabolisable energy calculated		MJ/Kg DM	10.1
Chloride	Wet	%	0.56
Sodium	Wet	%	0.20
Magnesium	Wet	%	0.20
Potassium	Wet	%	1.46
Calcium	Wet	%	1.06

* NIR (near infrared spectroscopy)

Table 3. Experiment 1. The effect of nutrition (low, medium and high diets) on superovulatory responses and cell numbers (mean ± SE) of blastocysts collected on day 6 (day 0= day of ovulation).

Treatment	Low	Medium	High
Final weight (kg)*	55.35±1.80^{a} (16)†	61.99±1.85^{b} (15)	63.12±1.85^{c} (15)
Weight change (kg)	-7.87±0.73^{a} (16)	-1.27±0.40^{b} (15)	-0.14±0.62^{b} (15)
No. corpora lutea	15.37±1.47 (16)	15.07±1.65 (15)	16.07±1.55 (14)
Blastocyst/corpora lutea	0.095 ± 0.04 (9)	0.04±0.03 (7)	0.18±0.04 (8)
Total cell no.	74.68±1.45^{a} (85)	66.43±1.29^{ab} (76)	62.01±0.84^{b} (85)
TE:ICM ratio	0.73±0.04^{a} (85)	0.70±0.04^{b} (76)	0.69±0.03^{b} (85)

*Final weight after adjusting for covariate (initial weight)

†Number in brackets is the total number of animals/embryos collected or stained

^{a,b} Values within rows with different superscripts are significantly different (P<0.05)

Table 4. Experiment 2. The effect of nutrition (low, medium and high diets) between day 6-12 before ovulation, day 0-6 before ovulation and between 0-6 after ovulation on superovulatory responses and cell numbers (mean ± SE) of blastocysts collected on day 6 (day 0= day of ovulation).

Treatment	HHH	MHH	MHL	MLH	MLL	LLL
Final weight (kg)*	64.62 ± 2.42 (13)[†]	62.61±1.93 (12)	58.58±1.78 (13)	60.27± 2.66 (14)	57.70± 1.77 (12)	54.94±1.95 (12)
Change in wt. (kg)	1.00± 0.61[a] (13)	-0.88± 0.63[a] (12)	-4.82± 0.82[b] (13)	-3.30± 0.84[a] (14)	-5.79± 0.77[b] (12)	-8.60± 1.06[b] (12)
No. corpora lutea	18.10± 2.63 (11)	17.75± 1.84 (10)	14.77± 2.92 (7)	13.88± 2.69 (9)	16.42±1.98 (8)	14.33± 2.49 (9)
Blastocyst/CL	0.16± 0.04 (129)	0.24± 0.03[a] (121)	0.16± 0.04[b] (96)	0.22± 0.05[a] (82)	0.10± 0.03[b] (78)	0.12± 0.03[b] (91)
TE: ICM ratio	0.723± 0.04[a] (88)	0.72±0.03[a] (93)	0.74± 0.05[b] (80)	0.72± 0.04[a] (63)	0.76± 0.05[b] (70)	0.76± 0.03[b] (88)
Total cell no.	74.01± 1.2[a] (88)	71.82± 1.11[a] (93)	85.08±1.85[b] (80)	70± 1.49[a] (63)	88.04±1.89[b] (70)	86.51±1.38[b] (88)

* Final weight after adjusting for covariate (initial weight)

[†] Number in brackets is the total number of animals/embryos collected or stained

[a,b] Values within rows with different superscripts are significantly different (P<0.05)

Table 5. Experiment 2. Partitioning the effect of diet into single degree of freedom comparisons.

	Final weight	Change in weight	Corpora lutea	Proportion embryos	Blastocyst/corpora lutea	Total cells	TE:ICM ratio
Diet (5 df) [†]	P < 0.001	P < 0.001	n.s	n.s	P = 0.039	P < 0.001	P < 0.001
Pre.Post (1 df)‡	P = 0.173	P = 0.17	NA	NA	P = 0.559	P = 0.269	P = 0.147
Pre (1 df) *	P = 0.003	P = 0.002	NA	NA	P = 0.285	P = 0.488	P = 0.210
Post (1 df) •	P < 0.001	P < 0.001	NA	NA	P = 0.006	P < 0.001	P < 0.001

[†] Diet is partitioned into three comparisons of interest, summarized below the dotted line.

‡ interaction between pre-mating and post mating (i.e. MHH = MHL = MLH = MLL).

* MHH, MHL versus MLH, MLL.

• MHH, MLH versus MHL, MLL.

n.s – not significant at the 5% rejection level

NA – partitioning not appropriate

DISCUSSION

This study has shown for the first time that short-term low nutrition imposed on superovulated ewes prior to artificial insemination increased the total number of cells in embryo and this increase was primarily due to enhance number of trophectoderm cells (Experiment 1). A subsequent experiment indicated that the nutritional signals enhancing embryo cell number was manifested during the post mating period rather than the pre-mating period. The lack of a difference between the total numbers of embryos produced by low, medium and high diets ewes (where the level of nutrition was markedly different), suggests that the extra intake observed in high group ewes was partitioned towards improving ewe body weight and thus condition rather than increasing ovulation or embryo growth. In contrast to spontaneously ovulating ewes where flushing (is the increase of nutrition 4 to 6 week before the mating) may enhance ovulation (Rhind et al., 1989), superovulatory responses were not compromised by restricted feeding in this study, presumably because individual potential was maximised as a result of gonadotrophic action. It must be stressed, however, that this occurred following short-term feed restrictions applied to ewes, which were in good body condition. A similar gain could not be expected following either prolonged feed restriction or the imposition of the present regimen on already under-nourished animals. The lack of a significant increase in ovulation rate and number embryos collected with the increase in food intake was unexpected and is at variance with previous reports (Smith 1985, 1988). Lindsay (1976) has described live weight as being 'a crude, inaccurate criterion which, because it describes only long-term changes in feeding, is incompatible with studies on many of the components of the reproductive process — which takes place over a few days or even hours'.

Dyck and Strain (1983) suggested that the critical window for nutritional effects on embryonal survival was early postcoital period. Our overall result shows that the critical window for nutritional effects on embryos quality in ewes could occur in the period immediately after mating. The results support previously published data of Nolan et al. (1998) and Negrao et al. (1997), who concluded that the yield of transferable embryos increased as the dietary energy intake decreased. In ruminant there is the risk that rapid shift in rumen fermentation caused by abrupt changes in diet composition and in the amount or indeed pattern of feed intake may temporarily disrupt metabolic homeostasis with consequent adverse effects on the embryo (McEvoy et al., 2001). Improved nutrition during the periovulatory period is generally regarded as beneficial to reproductive outcome at a naturally-occurring estrus but this does not appear to be the case when ewes are superovulated using exogenous gonadotrophins (McEvoy et al., 1995a,b, 2001; Lozano et al., 2000; Boland et al., 2001;) and beef heifers (Nolan et al., 1998; Yaakub et al., 1999).

Total cell number and the ratio of inner cell mass cells to total cells have been used as an indicator of embryo quality (Van Soom et al., 1997). The results of our present study shows that the number of the total cells were increased due to a greater proliferation of trophectoderm cells suggests that events associated with implantation and placental development may also be affected by these early events. In addition, culture conditions that caused a reduction in mouse ICM and total blastocyst cell number also reduced their long-term viability after transfer (Lane and Gardner 1997), and a deficient ICM cell number has been identified as a potential causative component of foetal growth retardation and large placenta, which are characteristic of the inherited BB/E diabetic rat model (Lea et al., 1996). Elevated glucose levels in vitro have been shown to retard the proliferation of rat blastocyst ICM and trophectoderm cell populations (De Hertogh et al., 1991) and, in the mouse, alter the metabolic state of the embryo (Moley et al., 1996). A number of peptide growth factors, expressed from either or both maternal and embryonic genomes, stimulate proliferation in the preimplantation embryo, which is mediated by signalling through their specific receptors (Kane et al., 1997). In relation to our maternal nutritional data, insulin and IGF family growth factors have been shown in vitro to stimulate preferentially ICM proliferation in early blastocysts (Kaye et al., 1992; Kane et al., 1997). Insulin is not expressed by the preimplantation embryo and maternally derived insulin is delivered to the ICM by receptor-mediated transcytosis across trophectoderm (Kwong et al., 2000).

Moreover, short-term exposure of preimplantation mouse embryos in vitro to insulin caused a long-term increase in foetal growth rate after transfer (Kaye and Gardner, 1999). In sheep the glucose metabolism is not critical for embryonic development, but beneficial at low concentrations and high concentrations can inhabit development, possibly by inhibiting tricarboxylic acid (TCA) cycle (Thompson et al., 1992). We conclude, therefore, that possibly the hyperglycaemic maternal environment generated by high nutrition provides the most likely mechanism to explain retarded preimplantation proliferation and reduced total cells especially trophectoderm cell number. Preimplantation embryos require uptake of amino acids to enhance protein synthesis for growth from the blastocyst stage (Lamb and Leese, 1994) and suboptimal exposure to amino acids reduced ICM and total cell number in blastocyst and rate of foetal development after embryo transfer (Lane and Gardner, 1997). The preimplantation embryo is particularly sensitive to epigenetic modifications that may have programming consequences (Reik et al., 1993; Dean et al., 1998), and our data strongly indicate, but not prove, that it is preimplantation embryo itself that is programmed. Some growth factors are also reported when added to culture system, can effect on the ICM cells, for example, LIF (cow, Margawati et al. 1997), TGFβ (cow, Marquant-Le Guienne et al. 1989), insulin (mouse, Harvey and Kaye 1990), IGF-1 (mouse, Harvey and Kaye 1992) and glutathione (cow, Kotaras and Seamark 1997). CSF-1 (mouse, Bhatnagar et al. 1995) and IGF-1 (buffalo, Narula et al. 1996) stimulate development of TE cells. Further investigations will explore the impact of dietary intake during superovulation on the production of transferable embryos including, determining the long-term outcome of changes in TE/ICM ratio. Available evidence indicate that the large offspring syndrome may also be explained as a consequence of metabolic stress to early embryos, raising the need for more caution in the introduction of new technologies to assisted conception in human (Leese et al., 1998). There is also a parallel between the effects on embryos mediated by high maternal diet in vivo in our study and the stress effects on embryos mediated by in vitro culture which can activate abnormal glycogen metabolism (Edirisnghe et al., 1984), free oxygen radical release (Johson and Nasr-Esfahani, 1994) and abnormal gene expression patterns (Eckert and Niemann, 1998; Wrenzyki et al., 2000). In addition the in vitro culture conditions appear to retard embryo compaction, which in turn reduces the allocation of cells to the ICM lineage and may lead to abnormal foetal and perinatal growth (Walker at al., 1996; Van Soom et al., 1997). During meiosis and fertilization, as genetic material divides and rearranges, it is exposed and open to mutation. A nutritionally unfavourable environment is a major risk factor (House, 2000). Thus, the consequences not only of in vitro culture but also maternal diet in vivo during the preimplantation period need to be addressed more rigorously in human and animal reproduction. Although downstream mechanisms responsible for propagating the early programming events into later gestation and postnatal life have yet to be determined, our results do emphasize the importance of periconceptional diet in the control of mammalian development, with clinical implications for long-term healthcare.

An association between increased feed intake, decreased plasma progesterone, and increased metabolic clearance of progesterone has been shown by Parr et al. (1982). It has been speculated that high dietary intake induces suppression of circulating progesterone during oocyte maturation which imparts a legacy of developmental retardation which leads to decreased embryo survival (McEvoy et al., 1995a; Robertson, 1995). Progesterone concentrations can be elevated by malnutrition and/or progesterone administration in the early stages (from day 3 to 6) in the sheep causes an increase in the weight of the fetus and its placenta (Kleemann, et al., 1994). Early evidence indicates that progesterone treatment changes cell linage differentiation in the sheep with differences in the proportion of ICM and TE (Hartwich et al., 1995). Because in the present study no progesterone measurements were performed, we can only speculate about the relation of progesterone concentrations with embryo cell numbers in this study. The reproductive tracts of a variety of species are known sites of IGF production (Pushpakumara et al., 2002). A particular feature of the IGF system is that the production of many of its components can be regulated by nutrition (McGuire et al., 1992; Roberts et al., 1997). This finding raises the possibility that metabolic status could alter oviductal activity via modulation of the IGF

system. Oviductal secretory proteins have been identified in many species including baboons (Verhage et al., 1989), cattle (Boice et al., 1990), goats (Abe et al., 1995), hamsters (Robitaille et al., 1988), humans (Verhage et al., 1988), mice (Kapur and Johnson, 1988), pigs (Buhi et al., 1990), rhesus monkeys (Verhage et al., 1997) and sheep (Sutton et al., 1984). It has been suggested that these proteins may have some effects on early cleavage-stage development. Thus, there is a possibility that any change in postovulatory progesterone concentration may affect early embryo quality or survival by modification of the oviductal environment, and this possibility is the basis of our next study.

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REFERENCES

Abe H, Onodera M and Sugawara S (1995) Immunological detection and characterization of an estrus-associated antigen in the goat oviduct *Journal of Experimental Zoology* **272** 134-141

Adams GP (1994) Control of ovarian follicular wave dynamics in cattle: implications for synchronization and superovulation *Theriogenology* **41** 19-24

Agricultural Research Council (1980) The nutrition requirements of ruminants livestock. *Slough, Commonwealth Agriculture Bureau.*

Assay RJ, Hyttel P, Roche JF and Boland MP (1994) Oocyte structure and follicular steroid concentrations in superovulated versus unstimulated heifers *Molecular Reproduction & Development* **39** 8-16

Bhatnagar P, Papaioannou VE and Biggers JD (1995) CSF-1 and mouse preimplantation development in vitro *Development* **121** 1333-1339

Blanchard T, Ferguson J, Love L, Takeda T, Henderson B, Hasler J and Chalupa W (1990) Effect of dietary crude-protein type on fertilisation and embryo quality in dairy cattle *American Journal of Veterinary Science* **51** 905-908

Boice ML, Geisert RD, Blair RM and Verhage HG (1990) Identification and characterization of bovine oviductal glycoprotein synthesized at estrus *Biology of Reproduction* **43** 457-465

Boland MP, Lonergan P and O'Callaghan D (2001) Effect of nutrition on endocrine parameters, ovarian physiology and oocyte and embryo development *Theriogenology* **55** 1323-1340

Buhi WC, Alvarez IM, Sudhipong V and Dones-Smith MM (1990) Identification and characterization of de novo-synthesized porcine oviductal secretory proteins *Biology of Reproduction* **43** 929-938

Butler WR (2000) Nutritional interactions with reproductive performance in dairy cattle *Animal Reproduction Science* **60-61** 449-457

Coop IE (1966a) The response of ewes to flushing. *World Review Animal Production* **4** 69

Coop IE (1966b) Effect of flushing on reproductive performance of ewes *Journal of Agricultural Science Cambridge* **67** 305-323

Dean W, Bowden L, Aitchison A, Klose J, Moore T, Meneses JJ, Reik W and Feil R (1998) Altered imprinted gene methylation and expression in completely ES cell-derived mouse fetuses: association with aberrant phenotypes *Development* **125** 2273-2282

De Hertogh R, Vanderheyden I, Pampfer S, Robin D, Dufrasne E and Delcourt J (1991) Stimulatory and inhibitory effects of glucose and insulin on rat blastocyst development in vitro *Diabetes* **40** 641-647

Dunne LD, Diskin MG, Boland MP, O'farrell KJ and Sreenan JM (1997) Nutrition and embryo survival in cattle *Irish Journal of Agricultural Food Record* **36** 95 abstract

Dunn TG and Moss GE (1992) Effects of nutrient deficiencies and excesses on reproductive efficiency of livestock *Journal of Animal Science* **70** 1580-1593

Dyck GW and Strain JH (1983) Post-mating feeding level effects on conception rate and embryonic survival in gilts *Canadian Journal of Animal Science* **63** 579

Eckert J and Niemann H (1998) mRNA expression of leukemia inhibitory factor (LIF) and its receptor subunits glycoprotein 130 and LIF-receptor-β in bovine embryos derived in vitro or in vivo *Molecular Human Reproduction* **4** 957-965

Edirisinghe WR, Wales RG and Pike IL (1984) Degradation of biochemical pools labeled with [^{14}C]glucose during culture of 8-cell and morula-early blastocyst mouse embryos in vitro and in vivo *Journal of Reproduction and Fertility* **72** 59-65

Gong JG, Bramley TA, Wilmut I and Webb R (1993) Effect of recombinant bovine somatotrophin on the superovulatory response to pregnant mare serum gonadotrophin in heifers *Biolology of Reproduction* **48** 1141-1149

Harding SA, Lane PW, Murray DA and Payne RW (2000) Genstat for Windows (5th Edition) Introduction Oxford: VSN International (204 pages)

Hardy AH, Handyside AH and Winston RML (1989) The human blastocyst: cell number, death and allocation during late preimplantation development *Development* **107** 597-604

Hartwitch KM, Walker SK, Owens JA and Seamark RF (1995) Progesterone supplementation in the ewe alters cell allocation to the inner cell mass *Proceeding of Australian Society of Medical Research* **26** 128

Harvey MB and Kaye PL (1990) Insulin stimulates mitogenesis of the inner cell mass and morphological development of mouse blastocysts *Development* **110** 963-967

Harvey MB and Kaye PL (1992) Insulin-like growth factor-1 stimulates growth of mouse preimplantation embryos in vitro *Molecular Reproduction and Development* **31** 195-199

House S (2000) Stages in reproduction particularly vulnerable to xenobiotic hazards and nutritional deficits *Nutrition and Health* **14** 147-193

Houseknecht KL, Boggs DL, Campion DR, Sartin JL, Kiser TE and Rampacek GB (1988) Effect of dietary source and level on serum growth hormone, insulin-like growth factor I, growth and body composition of beef heifers *Journal of Animal Science* **66** 2916-2923

Johnson MH and Nasr-Esfahani MH (1994) Radical solutions and cultural problems: could free oxygen radicals be responsible for the imprinted development of preimplantation mammalian embryos in vitro? *BioEssays* **16** 31-38

Kakar M A, Maddocks S and Walker SK (2001) Short-term nutritional effects before and after mating on embryo quality in sheep *Proceeding of Society for Reproductive Biology* 32 43 Abstract

Kane MT, Morgan PM and Coonan C (1997) Peptide growth factors and preimplantation development *Human Reproduction Update* **3** 137-157

Kapur RP and Johnson LV (1988) Ultrastructural evidence that specialized regions of the murine oviduct contributes a glycoprotein to the extracellular matrix of mouse oocytes *Anatomical Record* **221** 720-729

Kaye PL, Bell KL, Beebe LFS, Dunglison GF, Gardner HG and Harvey MB (1992) Insulin and IGFs in preimplantation development *Reproduction Fertility Development* **65** 373-386

Kaye PL and Gardner HG (1999) Preimplantation access to maternal insulin and albumin increases foetal growth rate in mice *Human Reproduction* **14** 3052-3059

Kleemann DO, Walker SK and Seamark RF (1994) Enhanced fetal growth in sheep administered progesterone during the first three days of pregnancy *Journal of Reproduction and Fertility* **102** 411-417

Kotaras PJ and Seamark RF (1997) Glutathione enhances bovine frozen-thawed sperm viability *Biology of Reproduction* **52** (Supplement 1) 132 abstract

Kwong WE, Wild AE, Roberts P, Willis AC and Fleming TP (2000) Maternal undernutrition during the preimplantation period of rat development causes blastocyst abnormalities and programming of postnatal hypertension *Development* **127** 4195-4202

Lamb VK and Leese HJ (1994) Uptake of a mixture of amino acids by mouse blastocysts *Journal of Reproduction and Fertility* **102** 169-175

Lane M and Gerdner KD (1997) Differential regulation of mouse embryo development and viability by amino acids *Journal of Reproduction and Fertility* **109** 153-164

Lea RG, McCracken JE, McIntyre SS, Smith W and Baird J (1996) Disturbed development of the preimplantation embryo in the insulin-dependent diabetic BB/E rat *Diabetes* **45** 1463-1470

Leese HJ, Donnay I and Thompson JG (1998) Human assisted conception: a cautionary tale. Lessons from domestic animals *Human Reproduction* **13** Supplement **4** 184-202

Lindsay DR (1976) The usefulness to the animal producer of research findings in nutrition on reproduction *Proceeding of Australian Society of Animal Production* **11** 217-224

Lozano JM, Boland MP and O'Callaghan D (2000) Effect of nutrition on embryo development in ewes *Proceeding of the 14th International Congress on Animal Reproduction, Stockholm* **1** 279

Mantovani R, Enright WJ, Keane MG, Roche JF and Boland MP (1993) Effect of nutrition and dose of follicle stimulating hormone (FSH) on superovulatory response in beef heifers In: *Proceeding A E T E* 234

Margawati ET, Pugh A, McDonald MF and Tervit HR (1997) Effect of leukaemia inhibitory factor (LIF) during maturation and culture on bovine blastocyst cell number, inner cell mass and trophectoderm cells *Biology of Reproduction* **56** (Supplement 1) 150 abstract

Marquant-Le Guienne B, Gerard M, Solari A and Thibault C (1989) In vitro culture of bovine egg fertilized either in vivo or in vitro *Reproduction Nutrition and Development* **29** 659-568

Maurasse C, Matton P and Dufour JJ (1985) Ovarian follicular development at two stages of an oestrus cycle in heifers given high-energy diets *Journal of Animal Science* **61** 1194-1200

McEvoy TG, Robinson JJ, Aiken RP, Findlay PA, Palmer RM and Robertson IS (1995a) Dietary-induced suppression of pre-ovulatory progesterone concentrations in superovulated ewes impairs the subsequent in vivo and in vitro development of their ova *Animal Reproduction Science* **39** 89-107

McEvoy TG, Robinson JJ, Aiken RP, Findlay PA and Robertson IA (1995b) Relationship between pre-ovulatory feed intake, progesterone priming and the subsequent in vitro development of ova collected from superovulated ewes *Theriogenology* **43** 276

McEvoy TG, Robinson JJ, Ashworth CJ, Rooke JA and Sinclair KD (2001) Feed and forage toxicants affecting embryo survival and fetal development *Theriogenology* **55** 113-129

McGuire MA, Vicini JL, Bauman and Veenhuizen JJ (1992) Insulin-like growth factor and binding proteins in ruminants and their nutritional regulation *Journal of Animal Science* **70** 2901-2910

Moley KH, Chi M M-Y, Manchester JK, McDougal DB and Lowry OH (1996) Alterations of intraembyonic metabolites in preimplantation mouse embryos exposed to elevated concentrations of glucose: a metabolic explanation for the developmental retardation seen in preimplantation embryos from diabetic animals *Biology of Reproduction* **54** 1209-1216

Narula A, Taneja M and Totey SM (1996) Morphological development, cell number, and allocation of cells to trophectoderm and inner cell mass of in vitro fertilized and parthenogenetically developed buffalo embryos: the effect of IGF-1 *Molecular Reproduction and Development* **44** 343-351

Negrao S, Nibart M and Humblot P (1997) Negative effects of overfeeding on superovulatory response and embryo production in dairy heifers *Proceeding 13th Scientific Meeting AETF Lyon Abstract*

Nolan R, O'Callaghan D, Duby RT, Lonergan P and Boland MP (1998) The influence of short-term nutrient changes on follicle growth and embryo production following superovulation in beef cattle *Theriogenology* **50** 1263-1274

O'Callaghan D and Boland MP (1999) Nutritional effects on ovulation, embryo development and the establishment of pregnancy in ruminants *Animal Science* **68** 299-314

O'Callaghan D, Yaakub H, Hyttel P, Spicer LJ and Boland MP (2000) Effects of nutrition and superovulation on oocyte morphology, follicular fluid composition and systemic hormone concentrations in ewes *Journal of Reproduction & Fertility* **118** 303-313

Parr RA (1982) Effects of maternal nutrition and plasma progesterone concentrations on survival and growth of the sheep embryo in early gestation *Journal of Agricultural Science* **98** 39

Patterson HD and Thompson R (1971) Recovery of inter-block information when block sizes are unequal Biometrika **58** 545-554

Pullman AL and Hughes AD (1986) RUMNUT User Guide, "An introduction to ruminant nutrition and the use of the Rumnut model. South Australian Department Agriculture, Turretfield, Rosedale, SA 5350

Pushpakumara PGA, Robinson RS, Demmers KJ, Mann GE, Sinclair KD, Webb R and Wathes DC (2002) Expression of insulin-like growth factor (IGF) system in the bovine oviduct at oestrus and during early pregnancy *Reproduction* **123** 859-868

Reik W, Romer I, Barton SC, Surani MA, Howlett SK and Klose J (1993) Adult phenotype in the mouse can be affected by epigenetic events in the early embryo *Development* **119** 933-942

Rhind SM, McKelvey WAC, McMillen S, Gunn RG and Elston DA (1989) Effect of restricted food intake, before and/or after mating, on the reproductive performance of Greyface ewes *Animal Production* **48** 149-155

Roberts AJ, Nugent RA, Klindt J and Jenkins TG (1997) Circulating insulin-like growth factors 1, insulin-like growth factor binding proteins, growth hormone, and resumption of estrus in postpartum cows subjected to dietary energy restriction *Journal of Animal Science* **75** 1909-1917

Robertson IS (1995a) Dietary-induced suppression of pre-ovulatory progesterone concentrations in superovulated ewes impairs the subsequent in vivo and in vitro development of their ova *Animal Reproduction Science* **39** 89-107

Robitaille G, St-Jacques S, Potier M and Bleau G (1988) Characterization of an oviductal glycoprotein associated with the ovulated hamster oocyte *Biology of Reproduction* **38** 687-694

Smith JF (1985) Protein, energy and ovulation rate. In 'Genetics of Reproduction in Sheep'. (Eds R. B. Land and D. W. Robinson) Butterworths: London pp 349-359

Smith JF (1988) Influence of nutrition on ovulation rate in the ewe *Australian Journal of Biological Science* **41** 27-36

Sutton JD, Hart IC, Broster WH, Elliot RJ, Schuller E (1986) Feeding frequency of lactating cows: Effects on rumen fermentation and blood metabolites and hormones *British Journal of Nutrition* **56** 181-192

Sutton R, Nancarrow CD, Wallace ALC and Rigby NW (1984) Identification of an oestrus-associated glycoprotein in oviductal fluid of the sheep *Journal of Reproduction and Fertility* **72** 415-422

Thompson JG, Simpson AC, Pugh PA, Tervit HR (1992) Requirement for glucose during in vitro culture of sheep preimplantation embryos *Molecular Reproduction and Development* **31** 253-257

Van Soom A, Boerjan M, Ysebaert MT and De Kruif A (1996) Cell allocation to the inner cell mass and the trophectoderm in bovine embryos cultured in two different media *Molecular Reproduction and Development* **45** 171-182

Van Soom A, Boerjan M, Bols PEJ, Vanroose G, Lein A, Coryn M and De Kruif A (1997) Timing of compaction and inner cell allocation in bovine embryo produced in vivo after superovulation *Biology of Reproduction* **57** 1041-1049

Verhage HG, Fazleabas AT and Donnelly K (1988) The *in vitro* synthesis and release of proteins by the human oviduct *Endocrinology* **122** 1639-1645

Verhage HG, Boice ML, Mavrogianis P, Donnelly K and Fazleabas AT (1989) Immunological characterization and Immunocytochemical localization of oviduct-specific glycoprotein in the baboon *(Papioanubis) Endocrinology* **124** 2464-2472

Verhage HG, Mavrogianis PA, Boomsma RA, Schmidt A, Brenner RM, Slayden OV and Jaffe RC (1997) Immunologic and molecular characterization of an estrogen-dependent glycoprotein in the rhesus (*Macaca mulatta*) oviduct *Biology of Reproduction* **57** 525-531

Voet D and Voet JG (1990) "Biochemistry" New York: Wiley & Sons, pp 297-298

Walker SK, Hartwich KM and Seamark RF (1996) The production of unusually large offspring following embryo manipulation: concepts and challenges *Theriogenology* **45** 111-120

Wrenzycki C, De Sousa P, Overstorm EW, Duby RT, Herrmann D, Watson AJ, Niemann H, O'Callaghan D and Boland MP (2000) Effects of superovulated heifer diet type and quantity on relative mRNA abundances and pyruvate metabolism in recovered embryos *Journal of Reproduction and Fertility* **118** 69-78

Yaakub H, O'Callaghan D and Boland MP (1997) Effect of dietary intake and glucose infusion on ovulation rate and embryo quality in superovulated ewes *Journal of Reproduction and Fertility* **19** 57 abstract

Yaakub H, Williams SA, O'Callaghan D and Boland MP (1999) Effect of type and quantity of concentrate on superovulation and embryo yield in beef heifers *Theriogenology* **51** 1259-1266