SHORT TERM EFFECTS OF DIFFERENT DIETS ON OVARIAN FUNCTION AND OOCYTE MATURATION OF RABBIT NULLIPAROUS DOES

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ABSTRACT

Determination of the nutritional needs in reproductive does has been intensively studied, but the direct influence of nutrition on the ovarian physiology is poorly known in rabbits. Some nutritional factors may influence oocyte maturation that is the first step affecting successful fertilization and preimplantational embryo development. In this context, leptin is a possible link between the nutritional status and the reproductive function. The goal of this work was to study the short term effects of feeding strategies during rearing period (from 11 to 16 weeks of age) on the endocrine and reproductive parameters of nulliparous rabbit does. A total of 40 New Zealand x California white rabbits were randomly allocated to two experimental groups (HL and SL) fed with rich fibrous diets with a high (HL: NDF 50% DM, ADL 16% DM) or a standard lignin content (SL: NDF 41% DM, ADL 5% DM). Ten does per group were euthanasized, five before (G1 group) and the other five (G2 group) after artificial insemination (AI). The remaining rabbits were used to measure the conception rate. Serum leptin concentrations, ovary and body weight, number of follicles ≥1 mm in size in the ovarian surface, ovulation rate, and blastocyst recovery rate were recorded. Cumulus oocyte complexes (COC) were aspirated from ovarian follicles ≥1 mm in size of one ovary and were matured *in vitro*. A total of 113 COC were treated progressively for cortical granules (CG) and nuclear staining and observed under a confocal laser-scanning microscope. Leptin levels were higher in SL than in HL group (5.49 vs. 4.53 ng/ml; $P<0.04$). However, the average weight of does (HL: 3526 g; SL: 3641 g) and ovaries (HL: 182.1 g, SL: 218.1 g), mean of total ≥1 mm follicles per ovary (HL: 7.3, SL: 5.8), and ovulation rate (HL: 14.4, SL: 16.6) were similar between nutritional groups at time of the first insemination (16 week). In addition, there were no significant differences in both nuclear and cytoplasmic *in vitro* maturation measured as metaphase II (HL: 67.5%, SL: 59.3%) and CG migration rate (HL: 26.1%, SL: 39.2%); blastocyst recovery rate (HL: 80.5%, SL: 71.0%), and fertility rate (HL: 75.5%, SL: 86.6%). In conclusion, both fibre-rich diet with low and elevated content of lignin during rearing period did not seem to exert any effect on the reproductive parameters of the nulliparous does.

Key words: Oocyte, Reproduction, Nutrition, Rabbit.

INTRODUCTION

Determination of nutritional needs of reproductive does has been extensively studied. During lactation, the feed intake of females increases very rapidly after kindling (60–75%). However, in primiparous does this increase is insufficient to cover the requirements due to maintenance and milk production. This results in a very negative energy balance when females are concurrently pregnant and lactating (Parigi-Bini *et al*., 1992). This situation largely explains the lower reproductive performance observed in primiparous females (Pascual *et al*., 1998). Several studies attempted to increase the feed intake during the first lactation by feeding the young females with a fibre-rich diet to improve their reproductive performance (Nizza *et al*., 1997; Xiccato *et al*., 1999). In the other way, plasma leptin

concentration is correlated with body lipid content and the index of body mass (Chilliard *et al*., 2005). Leptin signals the state of body maturity and inhibits reproduction when the level of body reserves is insufficient to sustain gestation or lactation (Moschos *et al*., 2002). The influence of leptin on ovarian function has been demonstrated in rabbits (Zerani *et al*., 2004).

The effects of nutrition on ovarian function have been reported in different species (O´Callaghan *et al*., 2000; Lozano *et al*., 2003; Adamiak *et al*., 2006; Ferguson *et al*., 2003; 2006; 2007). In cattle, energy intake restriction enhances subsequent *in vitro* development of oocytes (Mc Evoy *et al*., 1997), suggesting that nutrition may influence reproduction through oocyte maturation. Oocyte maturation implies both nuclear and cytoplasmic maturation (Yanagimachi, 1994) that is considered an important event for normal embryo development after fertilization (Yang *et al*., 1998). In mammalian oocytes, cortical granules (CG) migration has been used as a criterion for the assessment of cytoplasmic maturation. CG positioned just beneath the plasma membrane, render the oocyte ready for fertilization by triggering the cortical reaction, essential in the prevention of polyspermic fertilisation (Wang *et al*., 1997).

Since there is a relationship between body reserves status of animals and their reproductive success, the effect of fibre feeding strategy in nulliparous does at insemination time should be tested. However, there are no reports on the effects of nutrition on the ovarian function of rabbits. The aim of this study was to analyse the effects of two rich fibre diets with different lignin content given out during the rearing period (from 11 weeks to first AI) on serum leptin concentrations and both ovarian and reproductive parameters of nulliparous rabbit does.

MATERIALS AND METHODS

Animal and experimental design

A total of 40 nulliparous New Zealand x California white rabbits were used in this study. Rabbit does were held on the experimental farm at the Animal Production Department, Polytechnic University of Madrid (Spain) in individual flat-deck cages (16 h light per day). The animals were randomly allocated to one of two high fibrous dietary treatments with different quantity of insoluble fibre and lignin content: HL group: NDF (Neutro Detergent Fibre) 49.6% and lignin 15.5% of DM (dry matter); SL group: NDF 40.9% and lignin 4.9% of DM (Table 1). The feeding was supplied *ad libitum* during rearing period (from 11 weeks to first AI, at 16 weeks).

Table 1: Diet chemical composition (% DM)

	HL	SL
Dry matter	90.5	90.6
Ash	7.79	7.47
Energy (kcal/kg)	4.36	4.29
Crude Protein	16.6	16.8
EE (Ether extracts)	3.32	3.15
NDF (Neutro Detergent Fibre)	49.6	40.9
ADF (Acid Detergent Fibre)	33.9	20.2
ADL (Acid Detergent Lignin)	15.8	4.9

All the parameters were recorded at the end of the feeding period. Twenty animals were used for the study of fertility (number of parturitions per number of inseminations x 100). The other twenty were euthanasized to measure serum leptin concentrations, weight of does and ovarian parameters (n=10/group). In each group, five animals were euthanasized before AI (G1 group) and the other five after AI (G2 group).

Antral follicles in the ovarian surface and *in vitro* maturation (IVM) study were recorded in G1 groups, ovulation and embryo recovery rate in G2 groups. In the latter, AI was performed using a pool of fresh heterospermic semen with more than 20 million spermatozoa in 0.5 ml of a commercial

diluent (Magapor, S.L., Spain). Ovulation was induced by i.m. injection of 1 µg buserelin (Suprafact, Hoechst Marion Roussel, S.A., Madrid). The experimental procedures were approved by the Animal Ethics Committee of the Polytechnic University of Madrid (Spain) and were in compliance with the Spanish guidelines for the care and use of animals in research (BOE, 2005).

Blood sampling and hormone assay

Blood samples were collected by intra cardiac-puncture after euthanasia, were kept (room temperature) for 1 h and centrifuged (1200 g) for 10 min. Serum was then stored at –20ºC until assayed for leptin (n=10/dietary treatment). Leptin was determined in individual serum (100 µl) using a Multi-species Leptin Ria Kit (LINCO Research, Missouri USA). Intra and inter-assay coefficients of variation were 3.1% and 7.3%, respectively. The limit of detection after adjusting the standard curve to rabbit values was 0.1 ng/ ml HE (Human Equivalent).

Oocyte collection and *in vitro* maturation

Ovaries were recovered by laparotomy and ovary weight, number of follicles ≥1 mm in size in the ovarian surface, and ovulation rate were recorded. A total of 146 COC were obtained by aspirating with 2 ml syringe and 25G needle from ovarian follicles ≥1 mm in size of one ovary per animal under a microscope stereoscope. COC of each animal that had compact cumulus cells were washed and placed in 500 µl of maturation medium in four-well dishes and cultured for 16 h at 38°C under an atmosphere of 5% CO_2 in air with maximum humidity. The maturation medium was TCM-199 with 2 mM L-glutamine, 0.1 mg/ml sodium pyruvate supplemented with 10% fetal calf serum, 10 ng/ml epidermal growth factor, and 100 ng/ml insulin-like-growth factor. All products were purchased from Sigma (St. Louis, MO, USA) if not otherwise stated.

Confocal microscopy

After the maturation period, a total of COC (HL, n=47; SL, n=66) were treated for the confocal study. Firstly, cumulus cells were removed in 2 mM hyaluronidase by gentle pipetting. Next, oocytes were treated with 0.5% pronase to digest the zone pellucidae, fixed in PBS containing 4% paraformaldehyde and washed in PBS. Oocytes were then washed with 0.02% Triton X-100 and treated for 40 min with blocking solution (7.5% BSA). Oocytes were incubated 30 min at room temperature with 100 µg/ml FITC-LCA for GC staining and 15 min at 39ºC with 10 µg/ml Propidium Iodide for nuclear staining and were observed under a confocal laser-scanning microscope. According to Velilla *et al*. (2004) CG distribution was classified as follows: 1) homogeneous (CG distributed throughout the cytoplasm, being not cytoplasmic matured); 2) cortical (most CG distributed at the cortical area); 3) peripherycal (CG distributed adjacent to the plasma membrane forming a monolayer, being cytoplasmic matured).

Embryo recovery

A total of 74 embryos (HL, n=36; SL, n=38) were recovered at slaughter 3.5 days after AI by flushing the reproductive tract with PBS+0.3% BSA, from oviduct towards utero-tubal junction and uterine horn. Embryos were classified on the basis of conventional morphological criteria and according to their stage of development following the guidelines of the International Embryo Transfer Society.

Statistical analysis

ANOVA one-way statistical analysis was performed to study the differences in the mean weight of animals and ovaries, serum leptin levels, mean of ≥1mm follicles and ovulation rate between the experimental groups. Chi-square test was used to analyse the fertility, blastocyst recovery rate and to compare the nuclear maturation and CG migration index. The statistical analyses were performed using General Linear Model (GLM) procedure of SAS (SAS, 1998) and SPSS program.

RESULTS AND DISCUSSION

The mean weight of nulliparous does at first insemination was homogenous in accordance to the right body weight for the AI (around 80% adult body weight) to achieve good results. However, SL group showed higher body weight values than HL group, although not significant. Serum leptin concentrations were at the physiological range that could reflects the body maturity of the does to be inseminated, although significantly increased in SL ($P<0.04$) compared to HL group (Table 2). Our leptin results are in agreement with those of Ferguson *et al*. (2003) in pigs fed a high plane of nutrition; conversely, other works show that leptin levels were not influenced by dietary composition or feed intake (Adamiak *et al*., 2006; Ferguson *et al*., 2006). These conflicting results may reflect the differences between species and experimental conditions such as time of feeding or type of diet used.

Table 2: Mean concentrations of leptin, body weight and reproductive parameters in does fed with rich fibre diets with high (HL) and standard (SL) lignin content during rearing period

	Experimental Diet		
	HL	SL	n (animals/group)
Weight (g)	3526.1±91.0	3641.4±79.7	10
Leptin (ng/ml)	4.53±0.39a	5.49±0.42b	10
Fertility rate (%)	75.5±5.84	86.6±5.84	10
Ovary weight (g)	182.1±17.1	218.1±14.0	10
Follicles ≥1 mm per ovary	7.3±0.93	5.8±0.77	5
Ovulation rate per doe	14.4±2.65	16.6±1.32	5
MII rate (%)	67.5±7.50	59.3±9.63	5
CG migration rate (%)	26.1±6.86	39.2±8.72	5
Blastocyst recovery rate (%)	80.5±6.68	71.0±7.45	5

(a,b: $P<0.04$)

Among reproductive parameters (Table 2), HL showed lower ovaries weight and ovulation rate than their counterparts, whereas number of follicles ≥1 mm in size were higher in HL group, but differences didn't reach statistical significance. Ovaries weight and ovulation rate data agree with those found in gilts fed with high fibre diet by Ferguson *et al*. (2007), although they found fewer large follicles compared to control animals. In our study, the same body weight could explain the analogous ovary weight, mean of ≥1 mm follicles and ovulation rate between experimental groups (Table 2).

Oocyte maturation was measured as metaphase II (MII) rate and CG migration. In our experiment, nuclear and cytoplasmic maturation rate was similar on both groups (Table 2) although oocyte quality (cytoplasmic maturation rate) was higher in SL group. Also other studies have shown no differences in oocyte morphology, analysed by electron microscopy, between different nutritional treatments in ewes (O´Callaghan *et al*., 2000). In contrast, Ferguson *et al*. (2007) reported that fibre diet significantly improves oocyte maturity (MII) in gilts; however, gilts that received the fibre diet were heavier than control group, whereas in our experiment these differences were not significant. On the other hand, there is increasing evidence that the diet consumed before mating can have impact on embryo survival (Ashworth *et al*., 1999; Lozano *et al*., 2003), specially a high fibre diet (Adamiak *et al*., 2006; Ferguson *et al*., 2006; 2007). Conversely, our results showed similar blastocysts recovery rate at 3.5 day post insemination on both groups. According to the reproductive parameters measured, in this experiment the fertility rate of nulliparous does also showed better results in SL group (Table 2), although not statistically significant. So, in our experimental conditions, the high insoluble fibre diet with high lignin content (HL) could have similar influence on oocyte maturation, fertilization and preimplantational development process like the SL diet with lower lignin content similar to diets used in commercial farms.

CONCLUSIONS

In the present work, although there are differences in leptin serum levels, two high level of fibre in diets with different lignin content supplied during rearing period (from 11 weeks of age to first insemination), do not seem to influence the optimum reproductive performance normally obtained in rabbit nulliparous does.

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