World Rabbit Science Association
Proceedings 10th World Rabbit Congress – September 3 - 6, 2012– Sharm El- Sheikh –Egypt, 341- 345

EFFECTS OF FASTING DURING PREGNANCY IN RABBIT DOES

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ABSTRACT

This study sought to examine the effects of the food restriction performed in different stages of pregnancy on maternal leptin plasma concentrations and on the productive performance of both the rabbit does and their litters. A total of 40 primiparous non lactating New White Zealand females were randomly assigned into four groups according to their nutritional treatments during the pregnancy. Control group (C) was provided with 130 g/d of commercial food. The other three groups were fed with a restricted amount of food (30% restriction, 90 g/d) from day 0 to 10 day (R1), from day 9 to 18 (R2) and from day 19 to 28 of pregnancy (R3). At the end of the restriction period, the does returned to standard ration. Productive performance of R1 and control groups were comparable. The rabbits of R1 group showed a rapid increase in weight after the re-feeding and the lower values of leptin ($P<0.001$). This compensatory growth was not evident in other experimental groups. R2 recorded lower litter weight at weaning ($P<0.05$) and higher pre-weaning mortality than control group (26.98 *vs*. 16.85 %). R3 showed lower litter size ($P<0.01$), born alive ($P<0.01$), weight at kindling ($P<0.05$), litter weight at weaning ($P<0.05$) and higher perinatal (16.13 *vs*. 7.94%) and pre-weaning mortality (26.67 *vs*. 16.85 %) compared to control group. Milk production of the R3 was lower than in control ($P<0.05$). The effects of restricted feeding on pregnancy depend on the stage of privation during the pregnancy period: while a food restriction in early pregnancy did not seem to determine significant differences in productivity, a restriction in the last third of pregnancy, a critical period for energy homeostasis, resulted in the reduction of performance and impaired milk production. Food restriction reduced plasma leptin concentrations, in particular during the first period of pregnancy. However, the trend of leptin in pregnancy period and its relationship with body weight and fat reserves requires further study.

Key words: Rabbits, pregnancy, food restriction, reproductive performance, leptin.

INTRODUCTION

Nutritional disorders are known to reduce reproductive and productive performance (Rommers *et al.*, 2004a; Brecchia *et al.*, 2006). Diets with inadequate energy contents can induce both an excessive fatness and malnutrition of the does with subsequent reduction of the number and growth of newborns (Fortun-Lamothe and Lebas, 2006; Matsuoka *et al.*, 2006). A wide range of evidence indicates that the nutritional and hormonal environment during pregnancy influences not only fetal growth but also postnatal development (Godfrey and Barker, 2001). Indeed, the energy homeostasis appears even more complicated during pregnancy, when adaptive metabolic processes intervene and result in an increase of available energy either to supply the fetal growth or to increase fat storage utilized to support the future lactation. In commercial rabbit farm, the body condition and the energy balance of female are particularly critical because the does are generally inseminated during lactation and this could affect does and kits performance (Fernández-Carmona *et al*., 2000). It is important to find specific feeding plans to stimulate voluntary food intake during the late gestational period in the does which may be a feasible approach towards reduction of the energy deficit and improvement of performance (Castellini *et al.*, 2010). Several studies demonstrated that, after a restricted feeding, the appetite of rabbits improves and a sharp increase for voluntary feed intake occurs (Rommers *et al*., 1999). Moreover the rabbit can be used as an experimental model to elucidate the effects of anorexia during pregnancy on embryo-fetal development (Matsuoka *et al.*, 2006). A key hormone in energy homeostasis is leptin. Leptin is produced by adipose tissue and its blood levels are correlated with body reserves and the availability of nutrients (Ahima and Flier, 2000). Moreover, it is involved in the regulation of body weight either suppressing appetite or increasing metabolic rate (Friedman and Halaas, 1998). Leptin levels during pregnancy could affect placental and fetal development (Saylan *et al.,* 2011) but leptin biology varies markedly by species and its role is unclear (Ladyman *et al*. 2008). The aim of the present study was to examine whether food restriction during different periods of gestation alters body conditions and leptin concentrations in the mothers and the productive performance of their offspring.

MATERIALS AND METHODS

The trial was carried out at the experimental rabbit farm of the Department of Botany and Agri-environmental Biotechnology and Animal Science of the University of Perugia. The animals were housed individually in flat deck cages, the temperature ranged from +15 to +28 °C and the light schedule was of 16 L:8D. A total of 40 primiparous non lactating does (New White Zealand) of 6 months of age were induced to ovulate by injection of 10 µg of synthetic GnRH (Buserelin 0.8 µg) and then artificially inseminated according to the indications of Castellini and Lattaioli (1999).

The does were randomly assigned into four groups (10 does/group) according to their nutritional treatments. Control group (C) was fed with the standard ration of commercial food (130 g/d) from the day of IA until parturition. The other three groups were fed with a restricted amount of food (30% restriction, 90 g/d) based on the amounts recommended by Maertens (1993), from day 0 to 10 (R1), from day 9 to 18 (R2) and from day 19 to 28 of pregnancy (R3). At the end of the restriction period the does returned to standard ration.

Housing and experimental procedures were carried out according to recommendations of the IRRG (2005). At IA and on days 4, 8, 10, 14, 18, 22, and 26 of pregnancy, the does were subjected to: assessment of body weight (by electronic scale model Isolad - Vignoli - Forli, Italy); ultrasound scanning (ALOKA model SSD-500) of the perirenal regions to determine fat thickness and to estimate fat weight as described by Dal Bosco *et al.* (2003); body condition score (BCS), as described by Bonanno *et al*. (2005); blood sampling for leptin determinations as reported in a previous paper (Brecchia *et al.,* 2008). Leptin were determined by RIA, as reported elsewhere (Rommers *et al.,* 2004a). Twenty-four hours after birth, the number of suckling kits was adjusted to 7-10 per litter, and the pups were weaned at 26 d. Productive performance of the does (prolificacy, born alive and litter weight at kindling) and the litters (perinatal and pre-weaning mortality, litter size and weight at weaning) were assessed. Milk production was measured from day of parturition until to day 18 of lactation, by weighting the doe immediately before and after suckling. Data were scored and statistically analyzed by analysis of variance, two-tailed unpaired t test and a Chi-square test to assess the differences among proportions.

RESULTS AND DISCUSSION

As results from the trend of does body weight and perirenal fat weight of all groups (Figures 1 and 2), energy critical periods during gestation seem to be around day 10 and day 22, when occurs a rapid increase in energy requirements for the development of the gravid uterus (Fortun-Lamothe, 2006). The rabbits subjected to restriction during the first third of pregnancy showed a rapid increase in weight after the re-feeding with standard ration. This compensatory growth is not evident in other experimental groups, probably because the re-feeding corresponds to periods of pregnancy characterized by increased energy demands. The feed restriction in a stage of pregnancy where the energy requirements for the fetal growth are modest and the energy balance of the does is positive (Parigi-Bini *et al.,* 1990), could induce a subsequent compensatory increase in weight that allows rabbits to recover the lost weight. The data are in agreement with those reported by other Authors in non-pregnant female rabbits and re-fed *ad libitum* (Rommers *et al*., 2004b). Then, in addition to increasing the capacity of intake recommended for animals fed *ad libitum*, other physiological mechanisms may also intervene, determining the compensatory increase in weight. By analyzing the correlation between the various methods used for assessing body state, we can confirm the lack of relationship between weight and BCS reported by other Authors (Dal Bosco *et al.*, 2003; Cardinali *et al.*, 2008).

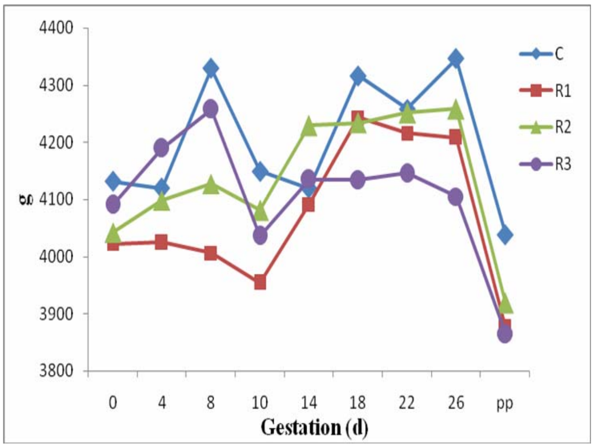

Figure 1: Body weight changes during pregnancy and on *post partum* day (pp)

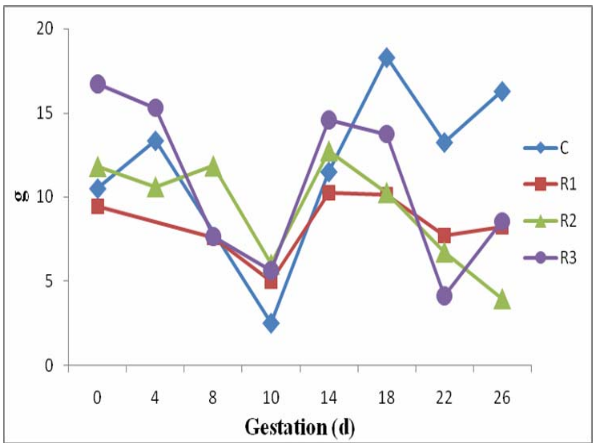

Figure 2: Perirenal fat weight changes during pregnancy

In a previous study we found that food restriction resulted in a decrease of plasma leptin concentrations in non pregnant rabbit does (Brecchia *et al.*, 2006). Leptin concentrations are also affected by feed restriction during pregnancy. Leptin levels were higher in the control group than R1 and R3 groups, respectively on the day 14 (P<0.05) and 26 (P<0.05) of gestation (Figure 3). R1 group showed lower average leptin concentrations during pregnancy (P<0.001) which may explain the anabolic effect for compensatory weight gain. The trend of leptin levels during food restriction performed in different periods of pregnancy showed a reduction of its concentrations in parallel with the food deprivation. Control group showed high leptin levels during the first part of pregnancy followed by a peripartum reduction. This trend is similar to that found in other species (Amico *et al.*, 1998; Block *et al.*, 2001; McFadin *et al.*, 2002; Ladyman *et al.*, 2008) in which hyperleptinemia is associated with hyperphagia and this paradox effect is called leptin resistance. In our study, feeding was controlled, we do not know if also in the rabbit, the hyperleptinenia is associated to hyperphagia as in other species. Thus, further studies are needed to evaluate the changes in leptin levels during pregnancy in rabbit does and if, as shown in other species, the phenomenon of leptin resistance occurs.

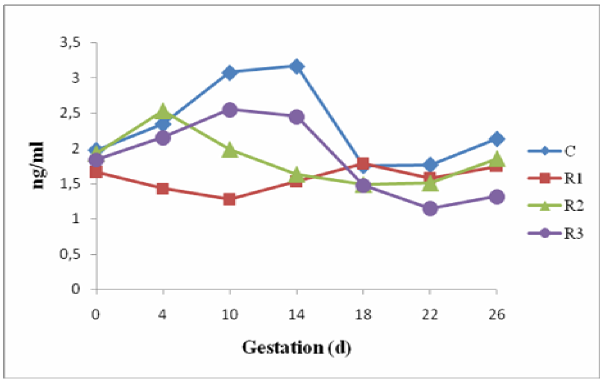

Figure 3: Leptin levels during pregnancy

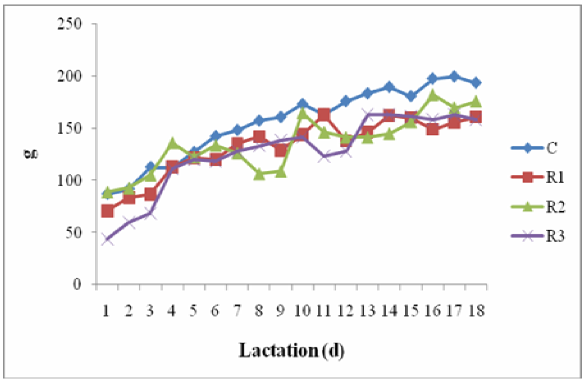

Figure 4: Milk production during pregnancy

Results of the effects of feed restriction during different pregnancy periods on productive parameters are shown in Table 2. Feed restriction in early gestation (R1) did not affect does and litter performance, as reported also by other authors (Rommers *et al.*, 2004b; Manal *et al.*, 2010) while feed restriction during mid gestation (R2) affects negatively the litter weight at weaning (P<0.05) and pre-weaning mortality than the control group. Compared to the control, reduced feeding levels during the last third of pregnancy (R3) had significant effects on litter size (P<0.01), weight at kindling (P<0.05), born alive (P<0.01), litter weight at weaning (P<0.05), and perinatal and pre-weaning mortality. The reduction in litter size was due to abortion of two does in group R3. In analogy with these data, also the milk production of the R3 group was significantly lower compared to control (P <0.05, Figure 4). Contrary to that reported in other species, there were no differences in the duration of pregnancy (Rossdale and Ousey, 2002).

Table 2: Performance of rabbit does

		C	R1	R2	R3
Durations of gestation	d	31.1±0.9	31.3±0.5	30.6±1.0	30.6±0.9
Litter size at kindling	n	9.8±2.3	9.0±1.7	9.3±4.1	6.2±3.4**
Born alive	n	9.5±2.5	8.3±1.8	9.1±4.3	5.2±3.1**
Litter weight at kindling	g	401.9±122.8	472.1±109.0	425.7±147.7	347.5±55.2*
Weight of individual kit	g	42.2±14.6	57.0±7.9	46.6±14.3	66.8±4.1
Perinatal mortality	%	7.9	7.9	2.7*	16.1***
Litter size at weaning[a]	n	7.4±2.0	6.4±1.1	6.6±2.5	5.5±1.7
Litter weight at weaning[a]	g	2890.0±574.7	2422.9±335.6	2095.7±446.0*	2204.8±292.9*
Pre-weaning mortality[a]	%	16.9	17.6	27.0***	26.7***
Weight of doe at weaning	g	4200.7±446.9	3946.4±311.0	4132.5±199.4	4426.7±224.1
Milk production	g/d	145.9±47.6	134.7±34.8	135.8±41.8	123.2±44.4**
Leptin during pregnancy	ng/ml	2.3±0.7	1.3±0.4***	1.7±0.9	1.8±0.6

***P<0.001; **P<0.01; *P<0.05 significant difference from control group (C)
a Calculating starting from the adjusted litter size.

CONCLUSION

Food restriction results in depletion of energy reserves but the effects of restricted feeding on pregnancy depend on the gestational period in which the privation occurs. As showed, the rabbit can quickly recover the weight lost, if the energy needs do not increase excessively, probably as a consequent of the anabolic effect due to the low concentrations of leptin. Thus, a food restriction in early pregnancy does not seem to determine significant differences in productivity, while a restriction in the last third of pregnancy, a critical period for energy homeostasis, induce a reduction of productive performance and an impaired milk production. The peculiar pattern of leptin in pregnancy and its relationship with body weight and fat reserves requires further study.

REFERENCE

Amico J. A., Thomas A., Crowley R.S., Burmeister L.A. 1998. Concentrations of leptin in the serum of pregnant, lactating, and cycling rats, and of leptin messenger ribonucleic acid in rat placental tissue. *Life Sci*, 63(16), 1387–95.

Ahima R.S., Flier JS. 2000. Leptin. *Annu Rev Physiol.;62:413-37.*

Block S.S., Butler W.R., Ehrhardt R.A., Bell A.W., Van Amburg M.E., Boisclair Y.R. 2001. Decreased concentration of plasma leptin in periparturient dairy cows is caused by negative energy balance. *J. Endocrinol*, 171, 341–50.

Bonanno A.,Mazza F., Di Grigoli A., Alicata M.L. 2005. Assessment of a method for evaluating the body condition of lactating rabbit does: preliminary results. *Proc. of the ASPA 16th Congr., Torino, Italy, June 28–30. Italian J. Anim. Sci., 4, Suppl. 2, 560.*

Brecchia G., Bonanno A., Galeati G., Federici C., Maranesi M., Gobbetti A., Zerani M,. Boiti C. 2006. Hormonal and metabolic adaptation to fasting: effects on the hypothalamic-pituitary-ovarian axis and reproductive performance of rabbit does. *Domest Anim. Endocrinol., 31(2), 105-22.*

Brecchia G., Cardinali R., Dal Bosco A., Boiti C., Castellini C. 2008. Effect of a reproductive rhythm based on rabbit doe body condition on fertility and hormones. *9th World Rabbit Congr. Verona – Italy, June 10-13, 309-313.*

Cardinali R., Dal Bosco A., Bonanno A., Di Grigoli A., Rebollar P.G., Lorenzo P.L., Castellini C. 2008. Connection between body condition score, chemical characteristics of body and reproductive traits of rabbit does. *Livest. Sci., 116, 209–215.*

Castellini C., Lattaioli, P. 1999. Effect of number of motile sperms inseminated on reproductive performance of rabbit does. *Anim. Reprod. Sci. 57, 111–120.*

Castellini C., Dal Bosco A., Arias-Álvarez M., Lorenzo P.L., Cardinali R., Rebollar P.G. 2010. The main factors affecting the reproductive performance of rabbit does: a review. *Anim. Reprod. Sci.,122; 174-82.*

Dal Bosco A., Castellini C., Cardinali R. 2003. Evaluation of body condition score in pregnant rabbit does by ultrasound scanner. *Proc. 15th Congr Naz. ASPA, Parma, Italy, 480-482.*

Fernández-Carmona, J., Pascual J.J., Cervera C. 2000. The use of fat in rabbit diets. *World Rabbit Sci,. 8(C), 29–59.*

Fortun-Lamothe L. 2006. Energy balance and reproductive performance in rabbit does. *Anim. Reprod. Sci., 93, 1–15*.

Godfrey K.M., Barker D.J. 2001. Fetal programming and adult health. *Public. Health Nutr., 4, 611–624*.

Friedman JM, Halaas JL. 1998. Leptin and the regulation of body weight in mammals. *Nature. 395; 763-70.*

IRGG (International Rabbit reproduction Group) 2005. Recommendations And Guidelines For Applied Reproduction Trials With Rabbit Does. *Word Rabbit Sci., 13, 147-164*.

Ladyman S.R. 2008. Leptin Resistance During Pregnancy in the Rat. *J. Neuroendocrinol. 20; 269–277*.

Maertens L. 1993. De voedingsbehoeften van konijnen voor de vleesproductie (Feed requirements for rabbits in commercial rabbit production). *CVB-documentatie rapport nr. 8*.

Manal A.F., Tony M.A., Ezzo O.H. 2010. Feed restriction of pregnant nulliparous rabbit does: consequences on reproductive performance and maternal behavior. *Anim. Reprod. Sci., 120, 179–186*.

Matsuoka T, Mizoguchi Y. Serizawa K., Ishikura T., Mizuguchi H., Asano Y. 2006. Effects of stage and degree of restricted feeding on pregnancy out come in rabbits. *J. Toxicol. Sci., 31(2), 166-175.*

McFadin E.L., Morrison C.D., Buff P.R., Whitley N.C., Keisler D.H. 2002. Leptin concentrations in periparturient ewes and their subsequent offspring. *J. Anim. Sci.,* 80(3), 738-43.

Parigi-Bini R., Xiccato G., Cinetto M. 1990. Energy and protein retention and partition in rabbit does during first pregnancy. *Cuni. Sci., 6, 19–29*.

Rommers, J.M., Kemp, B., Meijerhof, R., Noordhuizen, J.P.T.M., 1999. Rearing management of rabbit does. A review. *World Rabbit Sci., 7, 125–138.*

Rommers J.M., Boiti C., Brecchia G., Mejehof R., Noordhuizen J.P.T.M. 2004a. Metabolic adaptation and hormonal regulation in young rabbit does during long-term caloric restriction and subsequent compensatory growth. *Anim. Sci., 79, 255-264.*

Rommers J.M., Meijerhof R., Noordhuizen J.P.T.M., Kemp B. 2004b. The effect of level of feeding in early gestation on reproductive success in young rabbit does. *Anim. Reprod. Sci., 81, 151–158.*

Rossdale P. D., Ousey J. C. 2002. Fetal programming for athletic performance in the horse: Potential effects of IUGR. *Equine Vet. Educ., 14, 98–112*.

Saylan F, Köken G, Cosar E, Köken T, Saylan A, Arıöz DT, Sahin F, Köken R, Yılmazer M. 2011 Maternal and fetal leptin and ghrelin levels: relationship with fetal growth. *Arch. Gynecol. Obstet., 284; 327-9.*