# EFFECT OF A REPRODUCTIVE RHYTHM BASED ON RABBIT DOE BODY CONDITION ON FERTILITY AND HORMONES

**Brecchia G.[1], Cardinali R.[2], Dal Bosco A.[2], Boiti C.[1], Castellini C.[2]***

[1]Department of Biopathological Science, Hygiene of Animal and Food production, University of Perugia, Via S. Costanzo 4, 06121 Perugia, Italy
[2]Department of Applied biology, University of Perugia, Borgo XX Giugno 74, 06121 Perugia, Italy
*Corresponding author: cesare@unipg.it

## ABSTRACT

Productive protocols based on standard (Control: artificial insemination (AI) 11 days *post-partum*) or Conditioned rhythm (AI only when the does reach a perirenal fat depot weight estimated between 15 and 30 g) were compared. Two groups of fifty New Zealand White females were inseminated for seven consecutive cycles. The kits were weaned at 26 days. On the day of AI, all the does were submitted to ultrasound scanning of the perirenal regions to measure fat thickness and to BCS evaluation. Live weight, perirenal fat weight estimated and cumulative BCS were higher ($P<0.01$) in the Conditioned Group. These does, compared to the controls, showed higher sexual receptivity and fertility rate ($P<0.01$) as well as number of live born ($P<0.05$). The higher progesterone value found in control rabbits ($P<0.05$) confirms the negative effect of lactation on both sexual receptivity and fertility ($P<0.01$). Control does had lower ($P<0.05$) T3 blood concentrations, reflecting their worst body status. Multiparous does had higher ($P<0.01$) leptin levels, that only in Conditioned group reflected a greater body condition. Insulin and glucose concentrations were slightly increased in control does compared to conditioned rabbits. Further investigations are needed to analyze other important metabolites and hormones and to confirm the effect of body status condition on long-term performance of rabbit does. In conclusion, compared to fixed insemination at 11 days *post partum*, the conditioned rhythm seems more adapted to the reproductive physiology of rabbit does as testified by their higher sexual receptivity and fertility rates and better body condition. It is also evident that excessive fatness of non-pregnant does should be controlled by reducing feed ingestion during the dry period. Both BCS evaluation and measurement of perirenal fat by ultrasound technique permit to manage properly the energy balance and to improve body status, reproductive performance and welfare of rabbit does. Hormonal and metabolite analyses represent a good tool for understanding the physiological mechanisms required to meet these objectives.

**Key words**: Rabbit doe, Body condition, Rhythm of insemination.

## INTRODUCTION

Reproductive activity is under the control of the neuro-endocrine axis, but genetic, feeding, and management factors strongly modify hormonal release with consequent effects. The body condition and energy balance (EB) of female rabbits appears to be correlated to short- and long-term reproductive efficiency. Body fatness and fat mobilisation also affect the fertility rate (Castellini *et al.*, 2006). These two components are not independent since thin does will have less fat mobilisation during the subsequent lactation period. A notable effort has therefore been made to estimate *in vivo* body composition by using simple, non-destructive methods (Fortun-Lamothe *et al.*, 2002). Recently, ultrasound technique has been used to measure the perirenal fat thickness, which is the main reserve tissue of rabbits (Dal Bosco *et al.*, 2003; Pascual *et al.*, 2004). Body Condition Scoring (BCS) has also been proposed (Bonanno *et al.*, 2005; Cardinali *et al.*, 2008) as an alternative way for assessing the nutritional status of rabbit does.

Several metabolites, including glucose and non-etherified fatty acids (NEFA), and hormones, such as insulin and IGF-I, regulate ovulation rate, follicle development and embryo survival (Ashworth *et al.*, 1999; Comin *et al.*, 2002; Ferguson *et al.* 2003). Recently, also leptin has been implicated in several key points of the mammalian reproductive functions (Cunnigham *et al.*, 1999, Brecchia *et al.*, 2006). Blood level of leptin above a minimal threshold is necessary to activate the hypothalamus-pituitary-gonadal axis, trigger puberty and maintain normal reproductive function (Zerani *et al.*, 2005). Insulin was found to directly influence LH release by the anterior pituitary in rats (Weiss *et al.*, 2003) and glucose-induced insulin response is related to milk production (Sartin *et al.*, 1985).

The aim of this study was to investigate the long-term effect of a reproduction rhythm based on the body condition of rabbit does and to examine same blood parameters as specific markers of the body status.

## MATERIALS AND METHODS

### Animals and experimental design

The trial was carried out at the experimental rabbit farm of the Department of Applied Biology of the University of Perugia (Italy). The environmental temperature ranged from 15 to 28°C and relative humidity from 60 to 75%. One hundred New Zealand White nulliparous does were submitted to artificial insemination (AI) for seven consecutive cycles. Feed and water were provided *ad libitum.* The composition of feed was: crude protein 18.7%, crude fibre 14.7%, fat 4.8% and digestible energy 10.9 MJ/kg according to Maertens *et al.* (1988).
Does were divided into two groups according to the reproductive rhythm:

- Control group: AI at 11d *post partum*;
- Conditioned group: AI at perirenal fat depot between 15 and 30 g, according to Dal Bosco *et al.* (2007).

At AI, perirenal fat thickness by ultrasound scanning was measured, and BCS of loin and rump (Bonanno *et al*., 2005) was evaluated obtaining an aggregate value (from 0 to 4). The perirenal fat level of the conditioned does was estimated starting from 11d *post partum* by ultrasound scanning (Castellini *et al.*, 2006) at weekly intervals until they reached a suitable, predetermined, body fat to be artificially inseminated.

Weight at kindling and at weaning were also evaluated. Sexual receptivity was estimated by analysing the vulva colour and two classes were established: receptive (red or red-violet and turgescent) and non-receptive (whitish with non-turgescent vulva) following the IRRG recommendations (2005). AI was performed in the morning immediately after milking by inseminating 0.3 ml of diluted fresh semen, containing about 10 million sperms (Castellini *et al*., 1999).

Twenty-four hours after birth, the number of suckling kits was adjusted to 8 per litter, and the pups were weaned at 26 d, the kits were nursed once a day. The does that were not pregnant after three consecutive AI were eliminated.

### Biochemical Analyses

The blood samples were collected at the day of AI on the primiparous does and on sixth and seventh AI on the multiparous does from central ear vein, drawn into tubes containing EDTA, immediately centrifuged at 3000xg for 15 min and plasma stored frozen until assayed for hormones and metabolites. Insulin, leptin, total triiodothyronine (T3) were determined by RIA, as reported elsewhere (Rommers *et al*., 2004). Glucose was analyzed by the glucose oxidase method using the Glucose Infinity kit obtained from Sigma (Sigma Diagnostic Inc., St. Luis MO, USA). Progesterone concentrations in plasma samples was determined by radioimmunoassay, using specific antibody according to the procedure reported by Gobetti *et al*. (1992).

**Statistical Analysis**

Statistical analysis was done with mixed models adapted to repeated measures (Stata Corp. 2005) analyzing the fixed effect of reproductive rhythm and kindling order. Interaction was not showed in table because never significant.

## RESULTS AND DISCUSSION

Live body weight, perirenal fat weight estimated (Castellini *et al.*, 2006), and cumulative BCS at AI showed higher values in the Conditioned than in Control Group (Table 1). This better body condition can be explained by the longer mean remating interval (58.4 vs. 70.2 days). On the contrary, the Control group showed lower BCS, body and estimated perirenal fat weights likely due to the energy deficit occurring during lactation (Parigi-Bini *et al.*, 1998).

Compared to controls, the conditioned does, had higher ($P<0.01$) sexual receptivity and fertility rate. Such an improvement was expected given that the overlapping between lactation, known for reducing reproductive performance (Castellini *et al.*, 2006), and gestation and the resulting hormonal and energetic antagonism was virtually eliminated. The number of live born (Table 1) was higher ($P<0.05$) in does of the Conditioned group reflecting their better body condition, especially in multiparous ones, in according to Dal Bosco *et al*. (2007).

Recently, Cardinali *et al*. (2008) showed that 71.2% of does inseminated at 11d *post-partum* had poor BCS values, low sexual receptivity (37.2%) and low fertility rates (50.9%). An optimal body condition positively modulates the pituitary activity (FSH levels and preovulatory LH) and consequently the sexual receptivity and the fertility rate. Indeed, the higher ($P<0.05$) progesterone value of Control group (Table 1) confirms the negative effect of lactation on the reproduction function (Fortun-Lamothe *et al*., 1994).

The T3 plasma levels were lower ($P<0.05$) in the Control group reflecting the worst body status of rabbit does (Table 1). Brecchia *et al.* (2006) found that T3 blood concentrations decreased during fasting. Thus, the thyroid hormone concentration clearly reflects the energetic balance of the doe, and food deficiency reduces thyroid function, so that the animals could spare energy by decreasing adaptive thermogenesis (Brecchia *et al*., 2006). Mean plasma glucose and insulin concentrations were higher, although not significantly, in controls than in conditioned does (Table 1) suggesting that metabolic adaptation occurs between the two reproductive rhythm. Insulin controls intermediate metabolism and exerts an important role in ovarian function as well. Since there is evidence of active transfer of both insulin and leptin into the brain, these hormones could have a role in signaling the metabolic state of the animal (Woods *et al*., 2003).

Multiparous does showed an increase ($P<0.01$) of leptin level (Table 1), that only in Conditioned group reflecting the greater estimated perirenal fat weight ($P<0.05$) and cumulative BCS ($P<0.01$). Leptin receptors were detected in different ovarian structures of rabbits, including follicles at different stages of development, and the oviduct (Zerani *et al*., 2004, 2005), suggesting that leptin may regulate steroidogenesis of pre- and post-ovulatory follicles as well as fertilization and early embryonic development by proving a favorable local environment to gamete (sperm and oocyte) transport, sperm capacitation and oocyte maturation (Boiti, 2004).

**Table 1**: Effect of reproductive rhythm on reproductive and body traits and some blood parameters at AI

| | | Control | Conditioned | Control | Conditioned | Prob. | | Pooled SE |
|---|---|---|---|---|---|---|---|---|
| | nulliparous | primiparous | primiparous | multiparous | multiparous | group | parity | |
| Sexual receptivity (%) | 84.7 | 51.5 | 98.1 | 38.9 | 85.4 | ** | ** | 0.42 |
| Fertility (%) | 82.5 | 49.2 | 66.3 | 54.4 | 73.4 | ** | ** | 0.49 |
| Weight at AI (g) | 4020 | 3789 | 3965 | 3914 | 4090 | ** | * | 317 |
| Weight at kindling (g) | | 3741 | 3873 | 3847 | 3979 | ** | * | 280 |
| Weight at weaning (g) | 3850 | 3644 | 3680 | 3940 | 3976 | n.s. | * | 300 |
| Estimated perirenal fat weight (g) | 30.10 | 23.10 | 27.51 | 23.30 | 27.61 | * | n.s. | 11.55 |
| Cumulative BCS | 1.98 | 2.20 | 2.53 | 1.65 | 2.00 | ** | ** | 1.36 |
| Does in good body condition (%) | 85.0 | 41.2 | 81.3 | 45.2 | 84.3 | n.s. | n.s. | 0.49 |
| Progesterone (ng/ml) | | 0.71 | 0.46 | 0.69 | 0.35 | * | n.s. | 0.86 |
| T3 (nmol/l) | | 3.24 | 3.47 | 3.26 | 3.49 | * | n.s. | 0.79 |
| Leptin (ng/ml) | | 1.71 | 1.74 | 3.51 | 3.54 | n.s. | ** | 0.69 |
| Insulin (µU/ml ) | | 64.2 | 55.7 | 52.3 | 41.9 | n.s. | n.s. | 55.72 |
| Glucose (mg/dl ) | | 102.3 | 98.0 | 101.7 | 97.4 | n.s. | n.s. | 30.57 |
| Live born (n) | | 8.34 | 9.07 | 8.40 | 9.20 | * | n.s. | 3.18 |
| Mortality (%) | | 16.5 | 12.5 | 13.1 | 9.1 | n.s. | n.s. | 0.81 |

N= 780 AI; **P<0.01; * P<0.05

## CONCLUSIONS

Compared to fixed insemination at 11 days *post partum*, the conditioned rhythm seems more adapted to the reproductive physiology of rabbit does as testified by their higher sexual receptivity and fertility rates and better body condition. It is also evident that excessive fatness of non-pregnant does should be controlled by reducing feed ingestion during the dry period. Both BCS evaluation and measurement of perirenal fat by ultrasound technique permits to manage properly the energy balance and to improve body status, reproductive performance and welfare of rabbit does. Hormonal and metabolite analyses represent a good tool for understanding the physiological mechanisms required to meet these objectives.

## ACKNOWLEDGEMENTS

Research funded by PRIN 2005 prot. 2005070702_004.

## REFERENCES

Ashworth C.J., Beattie L., Antipatis C., Vallet J. 1999. Effect of pre- and post mating feed intake on blastocyst size, secretory function and glucose metabolism in Meishan gilts. *Reprod. Fertil. Dev., 11, 323-327.*

Boiti C. 2004. Underlying physiological mechanisms controlling the reproductive axis of rabbit does. *In*: *Proc 8th World Rabbit Congress. Puebla, Mexico.*

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. *In: Proc. 16th Congr. Naz. ASPA, 560.*

Brecchia G., Bonanno A., Galeati G., Federici C., Maranesi M., Godetti A., Gerani M., Boiti C. 2005. Hormonal and metabolic adaptation to fasting: effects on the hypotalamic-pituitary-ovarian axis and reproductive performance on rabbit does. *Dom. Anim. Endocrinol., 31(2), 105-22.*

Cardinali R., Dal Bosco A., Bonanno A, Grigoli A., Rebollar P.G., Lorenzo P.L., Castellini C. 2008. Body Condition Score, chemical characteristics of body and reproductive traits of rabbit doe. *Livest. Sci. (in press).*

Castellini C., Lattaioli P. 1999. Effect of number of mobilie sperm inseminated on reproductive performance of rabbit does. *Anim. Reprod. Sci., 57, 111-120.*

Castellini C., Dal Bosco A., Cardinali R. 2006. Effect of post-weaning rhythm on the body fat and performance of rabbit does. *Reprod. Nutr. Develop., 46, 195-204.*

Comin A., Gerin D., Cappa A., Marchi V., Renaville R., Motta M. 2002. The effect of an acute energy deficit on the hormone profile of dominant follicles in dairy cows. *Theriogen., 58, 899-910.*

Cunnigham M.J., Clifton D.K., Steiner R.A. 1999. Leptin's actions on the reproductive axis: perpectives and mechanisms. *Biol. Reprod., 60, 216-222.*

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

Dal Bosco A., Cardinali R., Mugnai C., Castellini C. 2007. Assessment of a reproduction rhythm based on the body condition doe: preliminary results. *In: Proc. 17th Congr. Naz. ASPA, Alghero, Italy, 560.*

Ferguson E.M., Ashworth C.J., Edwards S.A., Hawkins N., Hepburn M.G. 2003. Effect of different nutritional regimes before ovulation on plasma concentration of metabolic and reproductive hormones and oocyte maturation in gilts. *Repr. 126, 61-71.*

Fortun-Lamothe L., Bolet G., Lebas G. 1994. Influence of prolactin of foetal survival in rabbit does. *Anim. Repr. Sci., 37, 75-84.*

Fortun L., Lamboley-Gaüzére B., Banneliere C. 2002. Prediction of body composition in rabbit females using total body electrical conductivity (TOBEC). *Liv. Prod. Sci., 78, 133–142.*

Gobetti A., Zerani M., Bellini-Cardellini L. 1992. Relationship among mammalian gonadotropin-relasing hormone, prostaglians and sex steroids in the brain of crested newt, *Triturus carnifex. Prostaglandins, 44, 2809-218.*

International Rabbit reproduction Group 2005. Recommendations and guidelines for applied reproduction trial with rabbit does. *World Rabbit Sci., 13, 147-164.*

Maertens L., Moermans R., Groote G. 1988. Prediction of the apparent digestible energy content of commercial pelleted feeds for rabbits. *J. Appl. Rabbit Res., 11, 60-67.*

Parigi-Bini R., Xiccato G. 1998. Energy metabolism and requirements. *In: De Blas C., Wiseman J. (Eds) The nutrition of the rabbit, CABI Publishing, CAB International, Wallingford Oxon, UK.*

Pascual J.J., Blanco J., Piquer O., Quevedo F., Cervera C. 2004. Ultrasound measurements of perirenal fat thickness to estimate the body condition of reproducing rabbit does in different physiological states. *World Rabbit Sci., 12, 7–21.*

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

Sartin J.L., Cummins K.A., Kemppainen R.J., Marple D.N., Rabe C.H., Williams J.C. 1985. Glucagon, insulin, and growth hormone responses to glucose infusion in lactanting dairy caws. *Am. J. Physiol., 248, E108-114.*

StataCorp. 2005. Stata Statistical Software: Release 9.0 College Station, *TX: StataCorp*.

Zerani M., Boiti C., Zampini D., Brecchia G., Dell'Aglio C., Ceccarelli P. 2004. Ob receptor in rabbit ovary and leptin in vitro regulation of corpora lutea. *J. Endocrinol., 183, 279-288.*

Zerani M., Boiti C., Dall'Aglio C., Pascucci L., Maranesi M., Brecchia G., Mariottini C., Guelfi G., Zampini D. 2005. Leptin receptor expression and in vitro leptin actions on prostaglandin release and nitric oxide syntheses activity in the rabbit oviduct. *J. Endocrinol., 185, 319-325.*

Weiss J.M., Polack S., Diedrich K., Ortmann O. 2003. Effects of insulin on luteizing hormone and prolactin secretion and calcium signalling in female rat pituitary cells. *Arch. Gynecol. Obstetr., 269, 45-50.*

Woods S.C., Seeley R.J., Baskin D.G., Schwarts M.W. 2003. Insulin and the blood-brain barrier. *Curr. Pharm. Des., 10, 795-800.*