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Serum level of hormone and metabolites in pregnant rabbit does

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ABSTRACT - The aims of this study were to compare the hormones and metabolites serum levels and the reproductive performances of nulliparous (n=100) and primiparous pregnant does submitted to artificial insemination (AI) 11 days *post-partum*. On the day of AI, all the does were weighed and the sexual receptivity was evaluated. The kits were weaned at 26 day. Blood samples were collect by puncture of the marginal ear vein from one day before AI until few days before the kindling and assayed for hormones and metabolites. The higher sexual receptivity and the fertility in nulliparous than in primiparous does confirmed the negative effect of lactation. Nulliparous does showed higher blood concentration of leptine than primiparous, and in both the groups such level lowered during pregnancy, probably reflecting the reduction of the fat reserve. The insuline level increased during pregnancy in either groups as a consequence of the growing of the foetuses. In nulliparous does the cortisol, NEFA and T3 concentrations were higher than primiparous does. The glucose levels were similar in both the groups probably due to the homeostatic mechanisms controlling the glycemia. Hormonal and metabolite analyses represent a good tool for understanding the physiological mechanisms required to meet higher reproductive performance.

Key words: Rabbit does, Hormones, Metabolites, Performance.

Introduction - Commercial rabbit farms are used to artificially inseminate (AI) does 11 days after kindling. This protocol permits to improve the management, but it doesn't consider the reproductive physiology of rabbit does (Castellini *et al.,* 2003). The does cannot completely satisfy the high nutritional requirements during lactation that is exceptionally high (Pascual *et al*., 1999). Several hormones work together in linking growth, metabolism, energy homeostasis and reproduction functions (Hornick *et al*., 2000). Recent evidences suggest that leptin, synthesized by adipocytes (Zhang *et al*., 1994), is involved not only in the regulation of food intake, energy expenditure and metabolism (Barb, 1999) but also in control of reproductive functions. T3 blood concentration is a important key to analyze the metabolic adaptation and with the glucose level are good indicators of the energy balance. The NEFA (Non Esterificated Fatty Acids) concentration indicates mobilization of body lipids (Forthun-Lamothe, 2006). Insulin is a key player in the control of intermediary metabolism and exerts an important role in ovarian function (Brecchia *et al.,* 2005) and the role of corticosteroids are determinant of a normal pregnancy (Baldwin *et al.,* 1974).

The aim of this study was to analyze some hormones and metabolites serum level, as specific markers of the body status, in nulliparous and primiparous pregnant does.

Material and methods - The trial was carried out at experimental rabbit farm of Department of Applied Biology of the University of Perugia. One hundred nulliparous New Zealand White does of 5 months of age, housed individually under controlled conditions of light (14h light/10h darkness) and temperature (18-25°C), were artificially inseminated with 0.3 mL of diluted fresh semen, containing

about 10 million sperms (Castellini *et al.*, 1999). The positive primiparous does (n=80) were inseminated again 11 days *post-partum*. Sexual receptivity was estimated following the IRRG recommendations (2005). The weight of the does was recorded at AI and at weaning. Twenty-four hours after birth the number of suckling kits was adjusted to 8 per litter, the kits were nursed once a day and pups were weaned at 26 d. Blood samples were collected by puncture of the marginal ear vein starting the day before AI until 27th gestation day. Immediately after collection, blood samples were centrifuged at 3,000 *g* and plasma stored at –20°C until assayed for hormones and metabolites. Plasma leptin and insulin concentrations were determined by RIA using the multi-species leptin kit and porcin insulin kit, respectively (Linco Research Inc., St. Charles, MO, USA). The NEFA levels were determined by an enzymatic colorimetric method ACS-ACOD kit (Waco Chemical GmbH, Neuss, Germany). The glucose concentrations were determined by an enzymatic colorimetric method Glucose PAP kit while those of T3 were evaluated by the T3 RIA (C.T.) kit (Chematil s.r.l., Angri, SA, Italy). The cortisol levels were determined by RIA. Statistical analysis was done with mixed models (StataCorp., 2005), adapted to repeated measures. The basic model evaluated the fixed effect of parity order and days of gestation.

Results and conclusions – The lower sexual receptivity and fertility rate in primiparous does (Table 1) were expected given the overlapping between gestation and lactation, known for reducing reproductive performances (Castellini *et al.*, 2006). In pregnant nulliparous does the blood concentration of leptin was higher than in primiparous, and in both the groups its levels lowered during pregnancy, probably reflecting the reduction of the fat reserve (Table 2). In nulliparous does the cortisol, NEFA and T3 blood concentrations were higher than in primiparous does, maybe to ascribe the energy deficit caused by milk production, responsible for intense energy mobilization. The insulin levels increased during pregnancy in either groups as a consequence of the growing of the foetuses. The glucose levels did not show any significant variation in both the groups and this can explain the homeostatic mechanisms that controlling the glycaemia. Recently, Cardinali *et al*. (2008) showed that less than 1/3 of does inseminated at 11 days *post-partum* have a good body condition, and so it is not only hormonal parameters to influence reproductive performances but also body status.

In conclusion, comparing the hormones and metabolites levels in blood of nulliparous does (only gestation effect) and primiparous does (overlapping of gestation and lactation) it is possible to confirm that:

- leptin level is lower during gestation and the parity order has a little effect;
- NEFA level reflects the energy deficit in primiparous does;
- insulin and T3 increase during the gestation as a physiological reply to energetic requirement;
- cortisol level has a strong reduction in primiparous does to ascribe to milk production and body reserve mobilization.

Table 1. Performances of rabbit does.

		nulliparous	primiparous	Prob.	Pooled SE
Sexual receptivity (N=227)	%	74.6	37.3	**	3.2
Fertility (N=227)	"	80.2	46.8	***	1.5
Body weight A.I. (N=227)	g	4,133	4,045	n.s.	341
Live Born (N=127)	n	8.2	8.0	n.s.	2.6
Litter size at weaning (N=127)	"	7.0	6.0	*	1.4
Litter weight at weaning (N=127)	g	3,159	3,567	n.s.	665

****P<0.001; **P<0.01;*P<0.05; n.s.=not significant.*

Table 2. Effect of pregnancy day and parity order on same hormones and metabolites.

	1 d before AI		11 d gestation		21 d gestation		27 d gestation		Probability		Pooled SE
	null.	prim.	null.	prim.	null.	prim.	null.	prim.	days	parity	
Leptin (ng/ml)	4.10	3.47	3.67	3.04	3.28	2.65	3.05	2.42	***	*	1.72
Cortisol (ng/dl)	5.73	2.42	6.29	2.98	6.79	3.48	7.09	3.79	n.s	***	3.88
NEFA (nmol/L)	0.77	0.59	0.78	0.60	0.79	0.61	0.79	0.62	n.s.	***	0.97
T3 (nmol/l)	3.76	3.31	3.96	3.51	4.14	3.69	4.25	3.80	*	**	0.75
Insulin (µU/ml)	28.49	26.02	33.78	31.30	38.58	36.10	41.46	38.99	*	n.s.	26.82
Glucose (mg/dl)	112.7	117.6	111.7	116.7	110.9	115.9	110.4	115.4	n.s.	n.s.	19.2

*N=127 pregnant does; ***P<0.001; **P<0.01;*P<0.05; n.s.=not significant.*

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