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Effect of LPS-induced inflammatory state on some aspects of reproductive function of rabbit does

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RIASSUNTO – Effetto dell'infiammazione indotta con LPS su alcuni aspetti della funzione riproduttiva nelle coniglie. *Scopo della ricerca è stato quello di studiare un modello d'induzione sperimentale di infiammazione con lipopolisaccaridi (LPS) microbici nella coniglia fattrice e l'eventuale effetto sulla risalita degli spermatozoi. Due gruppi di 6 coniglie fattrici sono state inoculate per via intra-peritoneale rispettivamente con LPS di E. coli 0127:B8 (100 µg/kg peso vivo), o con soluzione fisiologica (controllo). Sono stati rilevati per 72 ore temperatura rettale e il numero dei leucociti; dopo inseminazione artificiale è stata valutata la risalita degli spermatozoi nel tratto riproduttivo femminile e la situazione ovarica. L'infiammazione sperimentale ha indotto un rilevante incremento della temperatura rettale e sostanziali modifiche a livello di leucociti che sono comunque scomparse entro 72 ore. Anche il numero di spermatozoi risaliti è stato significativamente più basso a livello di corna uterine e addirittura nullo a livello dell'ovidutto. In conclusione si può affermare che è possibile costruire un modello d'induzione dello stato infiammatorio nella coniglia mediante inoculazione intra-peritoneale di 100 µg LPS/kg di peso vivo.*

Key words: rabbit does, LPS, reproduction.

INTRODUCTION – The efficiency of the cycled production system in rabbit farms is greatly conditioned by the fertility rate of does. Nulliparous does generally exhibit high fertility rate (Castellini *et al.*, 1998), whereas the reproductive performances of multiparous does goes down. One reason of this reduction is related to the use of intensive reproductive rhythms which implies an overlapping between lactation and insemination which often produces a severe energy deficit. As in other mammals, lactation shows a strong hormonal antagonism with the reproductive activity. An other cause of hypo-fertility depends on the sanitary condition of does. Genital tract inflammation and/or infection is one of the major causes of infertility (Gram *et al.*, 2002) and often determined by incorrect practices of artificial insemination (AI). It has been demonstrated that uterine infection negatively affects fertility (Facchin *et al.*, 1999) and prolongs the life span of *corpora lutea* (Boiti *et al.*, 1999) due to uterine leukocytes infiltration, reduced prostaglandins synthesis and increased spermatozoa reabsorption. Lipopolysaccharides (LPS), constituents of the Gram-negative germ wall, are potent stimulators of prostaglandins synthesis and are widely used to simulate inflammation in several district and organs. The aim of the paper was to verify the effect of an LPS-induced inflammatory state on some aspects of reproductive function of non-lactating rabbit does.

MATERIAL AND METHODS – Two groups of 6 New Zealand White does (about 4 kg live weight, LW) were inoculated intra-peritoneally with 100 µg/kg LW *E. coli* LPS (0127:B8, Sigma-Aldrich) diluted in 2 ml of

saline or only saline (control). Blood samples (2 ml) were withdrawn through the marginal ear vein and white blood cells (WBC) number was evaluated by haemocytometer. Rectal temperature was measured with a copper-constantan thermocouple. Blood sampling and temperature detection were done just before 1, 2, 4, 8, 24, 48 and 72 h, following intra-peritoneal inoculation. After 60 h from treatment, AI was carried out inoculating close to the cervix region (about 18 cm depth) 0.2 ml of pooled semen containing 10×10^6 spermatozoa, having a progressive motility of 75%. Ovulation was induced by inoculation of 10 µg of synthetic GnRH. After 12 hours from AI, does were pharmacologically killed by an intravenous overdose of Tanax (Hoechst, Frankfurt). Successively, the female reproductive tract was excised and divided with haemostat clamps into cervixes, uterine horns and oviduct (Morton and Glover, 1974). Each tract was flushed with 5 ml of saline. The number of spermatozoa in the different tracts was counted after centrifugation of the recovered solution by a Thoma haemocytometer. Ovaries were immediately dissected and pre-ovulatory, mature and ovulated follicles were counted.

RESULTS AND CONCLUSIONS – LPS treatment induced an inflammatory status in rabbit does, mainly in the first 24-48 hours from inoculation with a relevant increase of body temperature (Figure 1). Leukocytes number (WBC/ml) showed a standard biphasic curve. After 1 h a marked decrease in WBC was observed, presumably due the high inflow of leucocytes in the inflamed district. Subsequently, this parameter increased for higher release by the production sites, surpassing the physiological level after 24 hours (Figure 2). After 72 h from LPS inoculation the original conditions of parameters were restored.

Our findings relating to temperature and WBC agree with Yamashiro *et al.* (1993) which showed, in rabbits inoculated intra-venous with LPS (5 µg/kg LW), a rapid increase of body temperature (about 1°C/hour), with a further increase until 3 hours; later the temperature reached a plateau and turned down until physiological level. The WBC curve showed the same trend of our experiment.

The intra-peritoneal LPS treatment did not show any effect on the ovary status (table 1) whereas the spermatozoa recovery of the LPS-treated does was significantly lower either in the uterine horn than in the oviduct. Morton and Glover (1974), in standard does artificially inseminated in the first vaginal tract, found in the oviduct quite the same spermatozoa recovery (from 1 to 0.3% of spermatozoa).

Several authors, in mice (Mackler *et al.*, 2003) and rabbit (Katsuki *et al.*, 1997), found that LPS enhanced macrophages activation and prostaglandin production which in turn increased the uterine contraction. Probably, according to Rozeboom *et al.* (2000), modifications of uterine environment and spermatozoa reabsorption are the major causes of the gametes transport failure.

In conclusion, our results showed that a systemic inflammation in rabbit does could be attained inoculating intra-peritoneal 100 µg LPS/kg LW. As expected, such moderate inflammation - restored in 72 h - reduced the number of spermatozoa capable to achieve the oviduct probably by activating and increasing WBC and spermatozoa reabsorption.

Obviously, inflammation affects many other aspects of the reproductive process: Kaushik *et al.* (2004) observed that LPS treatment in pregnant mice reduced the embryo implantation by altering leukocyte infiltration, degeneration of luminal glandular epithelium, and determining hyperplasia in various reproductive organs. Further research needs to verify the effect of local inflammation on some specific aspects of the female reproductive apparatus and on isolated spermatozoa.

Figure 1. Temperature variation in LPS or control does.

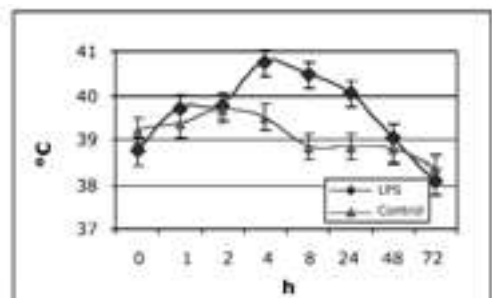


Figure 2. WBC variation in plasma of LPS or control does.

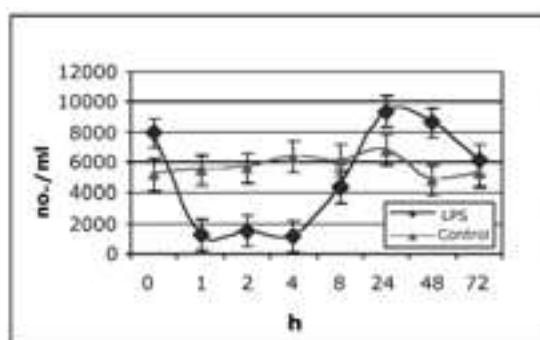


Table 1. Sperm recovery from different tract of genital apparatus and ovary status after 12 h from AI.

		Control	LPS	SDE
Sperm recovery				
Horns	%	9.33 ^b	0.43 ^a	0.45
Oviduct	%	1.41 ^b	0.00 ^a	0.05
Ovary status				
Pre-ovulatory follicles	n.	5.4	5.1	0.8
Ovulatory follicles	"	4.5	5.2	0.9
Haemorrhagic follicles	"	0.2	0.4	0.2

a, b: P<0.05.

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