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Effects of a bacterial lipopolysaccharide on the reproductive functions of rabbit does

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ABSTRACT

Systemic and local infections and inflammations are known to cause infertility in humans and animals. However, the mechanisms by which infection/inflammation induces infertility are only partially known. The objectives of this study were: (i) to provide models of systemic (acute) and local (sub-acute) inflammation by intra-peritoneal injection or intra-cervical deposition of lipopolysaccharide (LPS) in the rabbit and (ii) to assess their effects on uterine tissues and sperm transport in the genital tract of rabbit does.

Intra-peritoneal administration of different doses of LPS induced systemic effects such as fever, anorexia and changes in white blood cells (WBC) count. In our study, LPS inoculation (100 μg/kg) produced an inflammation-like status that lasted for about 3 days, with minimal distress for the animals. Intra-peritoneal administration of LPS 60 h before artificial insemination induced a rapid increase of IL-1β concentrations. The intra-cervical inoculation of LPS did not show any systemic effects, as confirmed by the lack of changes in body temperature, feed intake and WBC count. Histological examination of uterine tissues showed an endometritis-like inflammation status in LPS-treated does, more severe in those inoculated intra-cervically. The number of spermatozoa recovered from uterine horns and oviducts of intra-cervically treated does was less than that retrieved from intra-peritoneally treated animals and controls. These results suggest (i) that sub-acute or acute inflammation may cause infertility by compromising the uterine environment and/or impairing sperm transport and (ii) that the LPS-induced -infection/inflammation experimental model is useful for studying the mechanisms involved in reproductive dysfunctions in the rabbit.

1. Introduction

Asymptomatic, undiagnosed and untreated genital infections may have serious complications for reproductive health in both humans and animals. Sub-clinical uterine infections are very common in domestic animals, and can prove costly because of resultant infertility, increased culling for failure to conceive, reduced production, and expenditures for drug treatments (Laven and Peters, 1996; Boiti et al., 1999; Rosell and De la Fuente, 2009).

In rabbit farms, production efficiency is greatly conditioned by the fertility of does. Multiparous does generally exhibit much lower fertility rates than nulliparous does,

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due to intensive reproductive rhythms, energy deficit for the overlap between lactation and pregnancy, and poor health of the animals (Brecchia et al., 2006; Fortun-Lamothe, 2006). In fact, subclinical endometritis is often caused by incorrect artificial insemination (AI) practices (Boiti et al., 1999; Maes et al., 2008).

In females, microbial infections of the genital tract produce an inflammatory response characterized by the secretion of chemokines, cytokines and other mediators of inflammation by resident immune and non immune cells that drive the influx of neutrophils and macrophages in genital organs. The persistence of inflammation can affect the reproductive process by disrupting the uterine environment (Zerbe et al., 2003; Bridges et al., 2013) and vascularisation (Esteller Vico et al., 2007), increasing uterine contractions (Katsuki et al., 1997), impairing sperm vitality and transport (Urata et al., 2001; Brecchia et al., 2010), reducing embryo survival and implantation (Jaiswal et al., 2009) and inducing an endocrine switch from prostaglandin $F_{2\alpha}$ to E_2 (Herath et al., 2009a). Finally, the increase of the serum concentrations of LPS and cytokines can change the hormonal secretion of ovarian axis impairing the production of GnRH and LH, the growth and the functions of the follicles and corpora lutea, the time of ovulation and then the oestrus cycle (Battaglia et al., 2000; Karsch et al., 2002; Daniel et al., 2003; Williams et al., 2008). These effects are exerted by microbial proliferation and mediated through endotoxins, including lipopolysaccharide (LPS) that is present in the cell wall of gram-negative bacteria, which induce secretion of cytokines, and other mediators of inflammation by immune and non immune cells (Sheldon and Roberts, 2010; Swangchan-Uthai et al., 2012). Bacteria are detected by specific pattern recognition receptors on mammalian cells, called pathogen associated molecular patterns (Takeuchi and Akira, 2010). The most important group of these receptors is the Toll-like receptors (TLRs), a receptor family found in immune cells but also in the cells of other tissues (Davies et al., 2008; Sheldon and Bromfield, 2011).

Innate immunity relies on the detection of LPS by TLR4 in complex with accessory molecules as CD14, MD2 and MYD88 antigens on host immune cells (Park et al., 2009; Cronin et al., 2012). There is evidence that this receptor is also expressed in several cell types of the genital tract (Herath et al., 2007; Cronin et al., 2012; Krikun et al., 2012). Binding of LPS to TLR4 stimulates secretion of pro-inflammatory cytokines such as IL-1β, IL-6, IL-8 and TNF-α (Cronin et al., 2012; Herath et al., 2009b), which are regulators of acute phase responses such as fever, anorexia, and neuro-endocrine and behavioral alterations (Rothwell and Hopkins, 1995). Beside cytokines, LPS may increase the expression of several enzymes, such as inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2), which induce the generation of nitric oxide and highly toxic reactive oxygen species (ROS) in several immune and non-immune cells (Jana et al., 2009; Li et al., 2010). Moreover, activation of TLRs stimulates the release of chemokines that mobilize and activate immune cells (Pivarcsi et al., 2005; Cronin et al., 2012). At the local level, these mediators of inflammation affect the cellular, vascular and endocrine functions determining apoptosis, vascular disruption, tissue remodeling, and changes in hormonal secretion (Perfettini et al., 2000; Deb et al., 2005; Williams et al., 2008; Cotechini et al., 2014). Reproductive function may also be compromised by infection, inflammatory conditions and disease that do not directly involve the genital tract (Soto et al., 2003; Matalliotakis et al., 2010).

LPS is widely used to simulate *in vivo* and *in vitro* inflammation in several areas and organs (Harris et al., 2000; Williams et al., 2008; Sheldon and Roberts, 2010) and could be used to study the mechanisms by which infections and/or inflammation impair the reproductive system in rabbit. In addition, the rabbit is an excellent animal model for research, in particular for studying reproductive parameters (Cardinali et al., 2009; Polisca et al., 2010). Therefore, the aims of the present work were: (i) to provide models of systemic (acute) and local (sub-acute) inflammation by intra-peritoneal injection or intra-cervical deposition of LPS to study the reproductive dysfunctions in rabbit and (ii) to assess their effects on uterine tissues and sperm transport in the genital tract of rabbit does.

2. Materials and methods

The trial was carried out at the experimental farm of the Department of Applied Biology of the University of Perugia. Rabbits were exposed to a continuous photoperiod of 16 h light per day at 40 lx. Room temperature ranged from 18 to 27 °C. Fresh water was always available. Animals were fed with 130 g/day of a standard diet. All experimental procedures used in this study were approved by the Animal Ethics Committee of the University of Perugia (2013-049R) and were in compliance with the Italian guidelines for the care and use of animals in research. All efforts were made to minimize animal distress and to use only the number of animals necessary to produce reliable results.

2.1. Experiment 1

To establish the LPS dosage capable of inducing a systemic and acute inflammatory status, a preliminary dose–response study was performed. Fifteen healthy New Zealand White rabbit does of similar age (8 months) and weight (4 kg) were divided into five groups (3/group) and were inoculated intra-peritoneally with 25 (LPS25), 50 (LPS50), 100 (LPS100), and 150(LPS150) μg/kg body weight of *Escherichia coli* LPS (0127:B8, Sigma–Aldrich, St. Louis, MO, USA) dissolved in 2 mL of sterile saline, respectively, or with the same volume of saline (Control). The inflammatory response was assessed by measuring rectal temperature and number of white blood cells (WBC) just before and then 1, 2, 4, 12, 24, 48, and 72 h after LPS-treatment (time 0), while feed intake was recorded every 24 h. Rectal temperature was measured with a digital thermocouple (Vedolente, Artsana, Grandate, Como, Italy). Blood samples were drawn from the marginal ear vein using a 2.5 mL syringe provided with a 23 ga butterfly needle, and placed in pre-cooled plastic tube containing EDTA. The WBC number was immediately counted using a haemocytometer.