

Effect of unilateral, intraovarian infusions of bacteria on ovarian morphology in gilts

Barbara Jana, Aneta Andronowska, Jan Kucharski

Division of Reproductive Endocrinology and Pathophysiology, Institute of Animal Reproduction and Food Research of Polish Academy of Sciences, Olsztyn, Poland

[Received 7 December 2001; Revised 22 January 2002; Accepted 23 January 2002]

*The aim of this study was to investigate whether unilateral, intraovarian infusions of bacteria might have induced morphological changes in the contralateral ovary. Eleven sexually matured gilts with controlled estrous cycle were used. The animals were randomly divided into two groups: I (Gr. I, treated; n = 4), and II (Gr. II, control; n = 7). In Gr. I, 1 ml of bacterial suspension (10³ colony forming units/ml of saline of *Escherichia coli*, *Staphylococcus aureus* and *Corynebacterium pyogenes*, in proportion 1:1:1) was infused into the hilus of one ovary from the 15th to the 19th day of the estrous cycle. At the same time, 1 ml of saline was infused into the hilus of the contralateral ovary and into both ovaries of the control gilts. On the 7th day of the next cycle, the ovaries were dissected out. There were no significant differences in the number of follicles and corpora lutea (CL) as well as in weight and size between the bacteria-infused, contralateral and control ovaries. The microscopic observations of the bacteria-infused ovaries revealed the presence of focal infiltrations of neutrophils in the softened stroma, especially around dilated blood vessels filled with erythrocytes. In the contralateral ovaries, the number of regularly distributed neutrophils in the softened stroma was greater than that found in the bacteria-treated ovaries. CL of the bacteria-infused ovaries had more numerous, dilated blood vessels than CL observed in the contralateral gonads. More neutrophils were found in CL of both ovaries in Gr. I as compared to those observed in Gr. II. In Gr. II, single neutrophils were found also in the stroma where the tip of the cannula was inserted. This study revealed that in gilts, unilateral, intraovarian administration of bacteria did not change the number of ovarian structures, the weight and size of the bacteria-infused and contralateral ovary, but induced inflammatory changes in both ovaries.*

key words: ovarian morphology, bacteria, inflammation, gilts

INTRODUCTION

An illness often found in female mammals is inflammation of the ovaries (oophoritis). This pathological state more frequently occurs in women than in female animals. Oophoritis causes

usually many disturbances in the physiological rhythm of the menstrual or estrous cycle, leading to temporary or permanent infertility. A naturally occurring inflammation of the ovaries may result from spreading of inflammation from the uterus,

oviducts and utero-ovarian ligaments. The inflammatory process of ovaries is caused also by Gram-negative and -positive bacteria reaching the ovaries with blood or lymph, or as a result of intoxication. Viral infections caused by e.g. IBR and BVD-virus in cattle, and cytomegalovirus and mumps virus in women are also associated with oophoritis [3, 8, 10, 12]. Prolonged ovary inflammation may induce the simple chronic or cystic chronic oophoritis [6]. It has been found that, in gilts, infusions of *Escherichia coli* endotoxin (lipopolysaccharide, LPS), the active agent of Gram-negative bacteria, into the hilus of both ovaries induce the inflammatory process of the gonads [7].

Sometimes, the inflammatory process can refer to one ovary only. Our previous study revealed that, in gilts, infusions of LPS into the hilus of one ovary caused pathomorphological changes in both ovaries and deviations from normal plasma hormonal profiles. These disturbances partly depended on the location of LPS infusions [4]. We also demonstrated earlier that in gilts administration of bacteria into the hilus of one ovary caused significant changes in hormonal profiles, but these disturbances did not depend on the place of bacteria infusions [5]. However, it has not been studied as yet whether the inflammatory changed ovary following bacteria infusions can affect the morphology of the contralateral one.

To this purpose we estimated the numbers of follicles and corpora lutea, weight and size of ovaries in gilts after unilateral, intraovarian infusions of bacteria. Histological investigations of the ovaries were also performed.

MATERIAL AND METHODS

The study was performed on 11 crossbred gilts (Large White x Landrace) aged 9–10 months and weighing 90–100 kg. The animals were maintained in individual stalls under conditions of natural light and temperature. They were fed commercial grain mixture and tap water ad libitum. For this experiment only the gilts which exhibited two overt consecutive estrus were used. Estrous behaviour was observed in the presence of the boar-tester. Two days before the beginning of the study (the 13th day of the estrous cycle), during lateral laparotomy, polyvinyl cannulas (outer diameter 1.2 and inner diameter 0.8) were inserted into the hilus of each ovary and then located on the back of the gilt to infuse saline or bacteria. The gilts were randomly assigned to one of two groups. In group I (treated; n = 4), 1 ml of bacterial suspension (altogether 10³ colony forming

units/ml of saline suspension of *Escherichia coli*, *Staphylococcus aureus* and *Corynebacterium pyogenes*, in proportion 1:1:1) was infused into the hilus of one ovary from the 15th to the 19th day of the estrous cycle, twice a day (at 06:00 and 18:00). All bacterial strains — *Escherichia coli* (O25:K23/α:H₁) nr 1324, *Staphylococcus aureus* and *Corynebacterium pyogenes* nr 518 were obtained from the National Veterinary Research Institute, Department of Microbiology, Puławy, Poland. Simultaneously, 1 ml of saline only was infused into the contralateral ovary. The gilts of the control group (II; n = 7) received 1 ml of saline into the hilus of both ovaries on the same days of the estrous cycle. Every time, 1 ml of bacterial suspension or saline was infused into the ovary over 10 min. On day 7 of the next estrous cycle, the ovaries were dissected out and their weight and size were measured and the number of follicles and corpora lutea was counted. Afterwards, the ovaries were fixed in Bouin's fluid, embedded in paraffin and sectioned. The sections were stained with hematoxylin-eosin (H-E) and analysed under a light microscope (Nikon FXA). The diameter of the blood vessels' lumen was measured by employing the PC-IMAGE system (Foster Findlay Associates Ltd, UK). The mean (± SEM) number of ovarian structures (follicles, corpora lutea), weight and size of the ovaries as well as diameter of blood vessels' lumen were calculated for the control and the bacteria-treated gilts. The data were subjected to one-way analysis of variance (ANOVA) (Instat GraphPAD, San Diego, CA).

RESULTS

Infusions of the bacteria did not affect significantly the number of follicles and corpora lutea as compared to that found in the contralateral and control ovaries (Table 1).

The weight and size of bacteria-infused ovaries were similar to those determined for the contralateral and control ovaries (Table 2).

Light microscopic observations of the bacteria-infused ovaries revealed the presence of focal infiltrations of neutrophils in the softened stroma (Fig. 1). The analysis of the measurements of blood vessels' lumen showed statistically significant ($P < 0.01$) dilatation in both the ovaries after the bacteria administration in comparison to the control group (Table 1). Dilated blood vessels filled with erythrocytes and numerous neutrophils were observed in the bacteria-treated and in the contralateral ovaries (Fig. 2–4). In the softened stroma of the contralateral ovaries, more regularly distributed neutrophils

Table 1. Mean (\pm SEM) number of follicles, corpora lutea and diameter of blood vessels' lumen in ovaries of the bacteria-treated (B) and control (C) gilts. Different letters denote statistical significance: a, b — $p < 0.01$.

Parameter	Group	Bacteria- or saline-infused ovaries	Saline-infused ovaries
No. of follicles (from 2 to 8 mm in diameter)	B	9.23 \pm 1.87	9.81 \pm 0.44
	C	8.85 \pm 0.34	7.42 \pm 0.89
No. of corpora lutea (from 5 to 11 mm in diameter)	B	7.05 \pm 1.12	6.57 \pm 1.28
	C	6.71 \pm 0.52	7.71 \pm 0.72
Diameter of blood vessels lumen [mm]	B	0.44 \pm 0.04 ^a	0.41 \pm 0.05 ^a
	C	0.25 \pm 0.03 ^b	0.20 \pm 0.01 ^b

Table 2. Mean (\pm SEM) weight and size of ovaries in the bacteria-treated (B) and control (C) group

Parameter	Group	Bacteria- or saline-infused ovaries	Saline-infused ovaries
Weight [g]	B	4.82 \pm 0.81	5.15 \pm 0.72
	C	4.71 \pm 0.13	4.8 \pm 0.89
Size [cm ³]	B	5.09 \pm 0.16	5.25 \pm 0.21
	C	4.91 \pm 0.09	5.01 \pm 0.17

were found as compared to the bacteria-treated ovaries (Fig. 5). In the stroma of both ovaries after bacteria administration lymphocytes were found (Fig. 1, 4). Corpora lutea of the bacteria-infused ovaries had more numerous, dilated blood vessels than corpora lutea in the contralateral gonads (Fig. 6). In the bacteria-infused and contralateral ovaries, a greater number of neutrophils was found under the capsule and among the luteal cells in the whole of corpora lutea (Fig. 7) as compared to those observed in the control gonads (Fig. 8). In the ovaries of the control gilts, single neutrophils besides corpora lutea were also found in the stroma where the tip of the cannula was inserted.

DISCUSSION

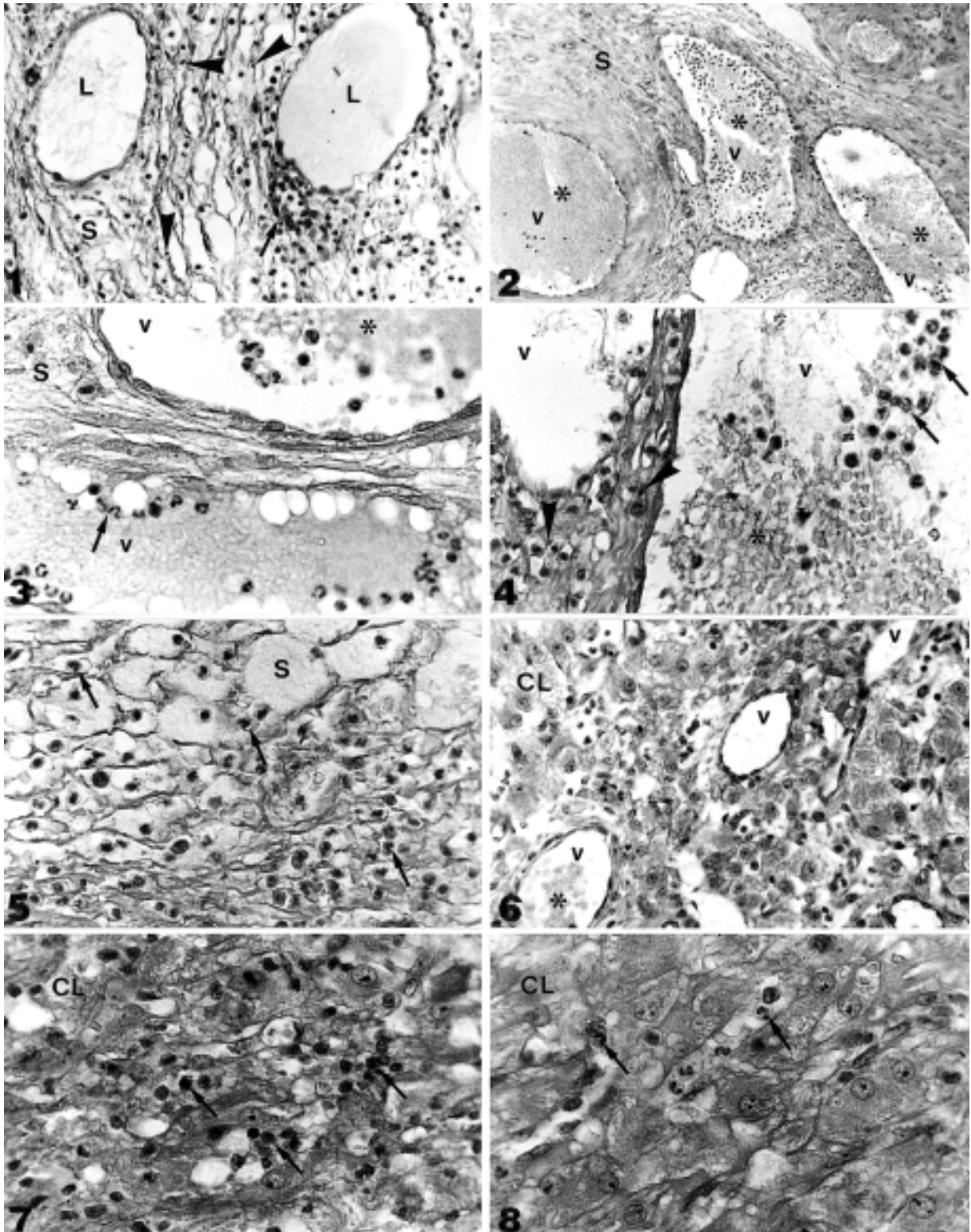
This is the first study designed to disclose whether the inflammatory changed ovary can affect the morphology of the contralateral one. Our experiment has demonstrated that in gilts, the unilateral, intraovarian infusions of suspension of Gram-negative and -positive bacteria did not induce significant changes in the number of follicles and corpora lutea as well as in weight and size of the bacteria-treated and contralateral ovaries. The histological assessment

of ovaries indicated the presence of inflammatory process in both ovaries.

In the available literature, there is a paucity of data dealing with the influence of inflammation provoked factors on the morphology of the porcine ovaries. The administration of LPS into the hilus of both ovaries caused insignificant changes in weight and size of the gonads, and the number of ovarian structures [7]. However, infusions of LPS into the hilus of one ovary in gilts caused a decrease in the number of follicles and corpora lutea as compared to those observed in the contralateral one, as well as cyst formation in both ovaries, resulting in an increase in the ovarian weight and size. However, the number of cysts in the LPS-treated ovaries was higher than that observed in the contralateral ones [4]. In the present study, no differences in the number of structures, weight and size between the bacteria-treated and contralateral ovaries were found. In this stage of the investigations, it is hard to point out reasons for such different responses of the gonads to the action of LPS and bacteria. The morphological differences can be partly explained by the various degree of hormonal disturbances observed after LPS and bacteria infusions [4, 5].

Microscopic observations revealed that in comparison to the control gilts, unilateral, intraovarian administration of bacteria suspension caused inflammatory changes (infiltrations of leukocytes, softening of the stroma, dilatation of blood vessels) in both the bacteria-infused and contralateral ovaries. It is known that LPS belongs to the group of factors having strong mitogenic effect on the lymphocytes. Therefore, it can be assumed that in our study lymphocytic infiltrations found in the ovarian stroma of bacteria-treated gilts probably resulted from the local LPS effect. The presence of the greater number of neutrophils in the corpora lutea and in the stroma of both bacteria-infused and contralateral ovaries as compared to the control group denotes also the stimulation of the immune cells system.

With regard to the present results, it is difficult to explain why the inflammatory changes were observed also in the contralateral ovary. It is possible that cytokines such as interleukin-1, interleukin-6, tumour necrosis factor- α , released by mast cells, lymphocytes and neutrophils in response to LPS, peptidoglycan and lipoteichoic acid (PG, LTA — cell wall components of Gram-positive bacteria) in the bacteria-treated ovary might reach the contralateral ovary via the circulation, affecting its function. It has been reported that *Staphylococcus aureus*, PG and LPS



Figures 1–8. H-E stained ovarian structures of the control and the bacteria-treated gilts. **1.** Focal infiltration of neutrophils in the bacteria-treated ovary ($\times 250$); **2, 3.** Neutrophils and erythrocytes in the dilated blood vessels in the bacteria-infused ovary ($\times 125, 500$, respectively); **4, 5.** Neutrophils in blood vessels (Fig. 4) and in the stroma in the contralateral ovary ($\times 500$); **6.** Dilated blood vessels in the corpus luteum of the bacteria-infused ovary ($\times 250$); **7.** Neutrophils in the corpus luteum in the bacteria-infused ovary ($\times 500$); **8.** Single neutrophils in the corpus luteum of the control gilt ($\times 500$); arrow — neutrophils, arrowhead — lymphocyte, asterisk — erythrocytes, CL — corpus luteum, L — lymphatic vessel, S — stroma, v — blood vessel.

induce expression of genes for main proinflammatory cytokines in human monocytes [13]. Fortunato et al. [2] have revealed that PG derived from beta hemolytic streptococcal cell wall and LPS stimulate cytokine release from human foetal membranes. Recent reports have emphasised the potential clinical importance of LPS and *Pseudomonas aeruginosa* as enhancers of mast cell-derived interleukins production in the course of inflammatory reactions and allergic diseases [1, 11]. Moreover, it cannot be excluded that LPS, PG and LTA also directly reach the contralateral ovary and stimulate proliferation of lymphocytes and cytokines production by cells of the immune system. This suggestion, however, should be verified by further experiments.

The present study revealed that in gilts infusions of Gram-negative and -positive bacteria into the hilus of one ovary from the 15th to the 19th day of the estrous cycle did not cause changes in the number of follicles, corpora lutea as well as size and weight of the bacteria-infused and contralateral ovary, but induced inflammatory changes in both ovaries.

REFERENCES

1. Boudreau RT, Garduno R, Lin TJ (2001) Protein phosphatase 2A and protein kinase C-alpha are physically associated and are involved in *Pseudomonas aeruginosa*-induced interleukin 6 production by mast cells. *J Biol Chem*, 12.
2. Fortunato SJ, Lombardi SJ, Menon R (1998) Immunoreactivity of human fetal membranes to peptidoglycan polysaccharide (PGPS): cytokine response. *J Perinat Med*, 26: 442–447.
3. Grooms DL, Brock KV, Pate JL, Day ML (1998) Changes in ovarian follicles following acute infection with bovine viral diarrhoea virus. *Theriogenology*, 49: 595–605.
4. Jana B, Kucharski J, Kotwica J (2000) Effect of unilateral, intraovarian infusions of *Escherichia coli* endotoxin on ovarian morphology and blood hormonal profiles in gilts. *Pol J Vet Sci*, 2: 105–110.
5. Jana B, Kucharski J, Kotwica J (2001) Blood hormonal profiles and ovarian morphology in gilts after unilateral, intraovarian infusions of bacteria. *Pol J Vet Sci*, 4: 187–192.
6. Kucharski J, Fitko R (1999) Influence of experimental oophoritis on ovaries morphology and sex hormone concentrations in rats and gilts. *Pol J Vet Sci*, 2: 113–117.
7. Kucharski J, Jana B, Zezula-Szpyra A (2001) Hormonal profile and morphological changes in pig ovaries after intraovarian infusions of *Escherichia coli* endotoxin. *Pol J Vet Sci*, (in press).
8. Miller J, van der Maaten M (1988) Experimentally induced infectious bovine rhinotrachitis virus infection during early pregnancy, effect on the bovine corpus luteum and conceptus. *Am J Vet Res*, 47: 223–228.
9. Norman RJ, Brännström M (1996) Cytokines in the ovary: Pathophysiology and potential for pharmacological intervention. *Pharmacol Ther*, 3: 219–236.
10. Sharma T, Nadasdy T, Leech R, Kingma D, Johnson L, Hanson-Painton O (1994) In situ hybridization study of "primary" cytomegalovirus (CMV) oophoritis. *Acta Obstet Gynec Scand*, 73: 429–431.
11. Stassen M, Muller C, Arnold M, Hultner L, Klein-Hesseling S, Neudorfl C, Reineke T, Serfling E, Schmitt E (2001) IL-9 and IL-13 production by activated mast cells is strongly enhanced in the presence of lipopolysaccharide: NF-kappa B is decisively involved in the expression of IL-9. *J Immunol*, 166: 4391–4398.
12. Taparelli F, Squadri F, De-Rienzo B, Lami G, Fornaciari A (1988) Isolation of mumps virus from vaginal secretion in association with oophoritis. *J Infect*, 17: 255–258.
13. Wang JE, Jorgensen PF, Almlof M, Thiemeermann C, Foster SJ, Aasen AO, Solberg R (2000) Peptidoglycan and lipoteichoic acid from *Staphylococcus aureus* induce tumor necrosis factor alpha, interleukin 6 (IL-6), and IL-10 production in both T cells and monocytes in a human whole blood model. *Infect Immun*, 68: 3965–3970.

