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CORE

# Ovarian morphology and hormonal profiles in gilts following surgical denervation at day 12 of the oestrous cycle — preliminary data

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[Received 17 September 2003; Accepted 23 October 2003]

To establish the role of the nervous system in the etiopathogenesis of ovarian cysts we studied ovarian morphology and hormonal profiles in gilts that had undergone bilateral surgical denervation at day 12 of the oestrous cycle. Blood samples were collected from day 13 of the first cycle until day 20 of the second studied cycle. On this day the number of follicles (6 to 10 mm in diameter) was lower (p < 0.01) in the denervated ovaries when compared to the control group. Denervation resulted in a dramatic reduction in the number of TH/D $\beta$ H- and/or NPY-IR nerve fibres, especially in the vicinity of the follicles. Furthermore, denervation lead to a decrease (p < 0.05-0.001) in plasma concentrations of  $P_4$  (at day 15 and 16 of the first cycle and during the luteal phase of the second studied cycle) and LH and  $E_2$  (mainly during the perioestrous period). These results suggest that sympathetic neurons supplying ovaries may participate in the follicular development and steroidogenesis in gilts.

key words: denervation of ovaries, ovarian morphology, hormones, gilts

## INTRODUCTION

The role of the nervous system in the control of normal ovarian secretory activity and follicular development has already been documented in part. However, only little attention has so far been given to the possibility that a derangement in neurogenic inputs may be an underlying component of some ovarian pathologies such as cystic ovarian disease (COD), leading even to infertility. It is generally assumed that this pathological state is caused mainly by disturbances in the function of the hypothalamic-pituitary-ovarian axis, causing impairment to the synthesis, release, and/or storage of various hormones of this functional unit. However, it has been suggested that an alteration in the activity of the sympathetic neurons innervating the ovary may contribute to the etiology and/or progression of cysts in rats [5]. In human polycystic ovaries an increased density of sympathetic nerves has been found and wedge resection, especially when compromising the hilus, has been shown to be effective in COD patients unresponsive to hormonal treatment [4]. To establish the putative role of the nerves in the etiopathogenesis of ovarian cysts, we studied ovarian morphology and hormonal profiles in gilts subjected to ovarian denervation.

## **MATERIAL AND METHODS**

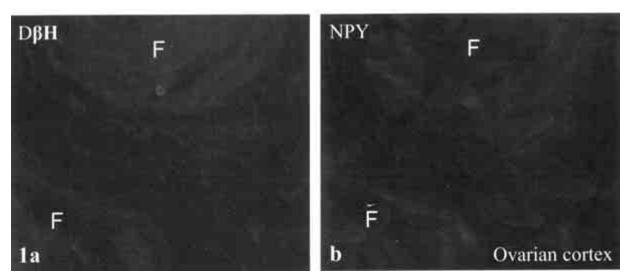
On day 12 of the oestrous cycle a cannula was inserted into the jugular vein in the gilts of the control and experimental groups (n = 6 each). Next a median laparotomy was performed in the experi-

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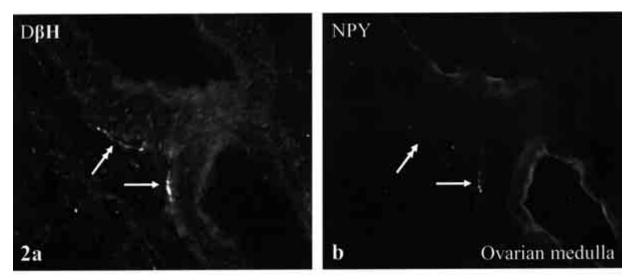
mental group and both the plexus and superior ovarian nerves were transected bilaterally. Blood samples were collected from day 13 of the first cycle until day 20 of the second studied cycle. Plasma LH, P<sub>4</sub>, and E<sub>2</sub> levels were determined by RIA. After the last blood sample collection the ovaries were dissected out and the number of follicles was counted. Cryostat sections of the ovary from all the animals were studied by means of a routine double-immunofluorescence technique in order to establish the intraovarian distribution and co-incidence pattern of TH, D $\beta$ H and/or NPY. The mean (±SEM) number of follicles was calculated per ovary and the data were subjected to one-way analysis of variance (ANOVA). The diurnal hormone levels were assessed by analysis of variance for two-factorial, orthogonal repeated measures. The Newman-Keuls test was applied for calculation of the statistical significance of mean differences (GraphPad, Prism, USA).

#### **RESULTS AND DISCUSSION**

After neurectomy, the number of ovarian follicles of 6 to 10 mm in size was lower than that found in the control gilts  $(2.3 \pm 0.5 v. 7.3 \pm 0.4, p < 0.01)$ . Double-immunofluorescence revealed a dramatic decrease in the number of TH/D $\beta$ H and/or NPY nerve terminals in the denervated gonad, especially in the vicinity of the ovarian follicles (Fig. 1). However, transection of both nerve trunks was unable to cause total denervation, as after this procedure sympathetic perivascular nerve terminals were still visible within the ovary (Fig. 2). Denervation resulted also in a re-



**Figure 1.** Complete disappearance of D $\beta$ H- (**A**) and NPY-IR (**B**) nerve fibres after transection of the superior ovarian and plexus nerve in the pig. F — follicle;  $\times$  200.



**Figure 2.** Scarce perivascular  $D\beta$ H/NPY-IR (single-headed arrow) and  $D\beta$ H-IR but NPY-immunonegative (double-headed arrow) nerve terminals around an artery in the ovarian medulla after transection of the superior ovarian and plexus nerve in the pig;  $\times$  200.

duction (p < 0.05–0.001) of E<sub>2</sub> and LH plasma levels during the perioestrous period and days 18–20 and 19–20 of the second cycle, respectively. Furthermore, the plasma P<sub>4</sub> level in gilts from the experimental group was lower (p < 0.05–0.001) on days 15–16 of the first and 3–16 of the second cycle, when compared to control values.

In denervated ovaries both the number of large follicles and the density of TH/D $\beta$ H- and/or NPY-IR fibres was reduced. This is in agreement with an earlier study, revealing that the chemical sympathectomy of ovaries in guinea pigs also caused a reduction in the number of large follicles [1]. It should be stressed that the occurrence of some nerve terminals within surgically denervated gonads, as observed in the present study, strongly suggested that the porcine ovary received part of its nerve supply from the pelvic ganglia. After neurectomy we observed a decrease in plasma P<sub>4</sub> level on days 15–16 of the cycle. A drop in P<sub>4</sub> concentration was previously found after pharmacological blockade of ovarian  $\beta$ -receptors in cows during the luteal phase of the oestrous cycle [2]. In the present study the secretion of E<sub>2</sub> and LH was decreased in the perioestrous period in the gilts with denervated ovaries, as in rats [3]. In our experiment a decrease in the E<sub>2</sub> level during

the perioestrous period probably caused a lack of the preovulatory LH surge and ovulation. Moreover, taking into consideration a decreased  $P_4$  level during the second studied cycle in gilts after neurectomy, it is obvious that luteinisation did not occur either. A disruption of cyclicity was also observed in rats after ovarian denervation [3]. The above data confirm the important role of the nervous system in follicular development and in the secretory function of the ovaries in gilts.

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