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The morphometric parameters of adrenal cortex in sows: in normal condition and after prolactin infusion

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The aim of this study was to examine the effect of experimental hyperprolactinemia on the sterological parameters of porcine adrenal cortex. In cyclic sows, after preovulatory luteinizing hormone (LH) peak, porcine prolactin (PRL, 0.3 mg) or saline were administered iv. for 48 h at 2 h intervals. Next sows were slaughtered and adrenal glands were dissected. Stereological analysis of the left adrenal gland did not reveal any significant differences between control and PRL-treated sows. Experimental hyperprolactinemia did not affect the volume of particular cortical zones, the number and the volume of adrenocortical cells or the average volume of their cell nuclei. Moreover, we present for the first time a detailed stereological description of adrenal cortex in sows.

key words: prolactin, stereology, adrenal cortex, sows

INTRODUCTION

Adrenal cortex steroidogenesis is under multihormonal regulation with the well-established stimulatory role of ACTH. Adrenocortical steroid secretion may also be controlled by prolactin (PRL) acting through its adrenal receptor [8]. There is in vivo and in vitro evidence that PRL affects adrenal steroidogenesis in guinea pigs [17], rats [1], cows [5], baboons [16], and humans [4, 18]. In vitro studies performed on rats and guinea pigs have shown that PRL stimulated adrenal secretion of corticosterone [15], androstenedione, dehydroepiandrosterone (DHEA) and cortisol [14]. The release of DHEA and/or dehydroepiandrosterone sulphate (DHEAS) by adrenals was also elevated in the presence of PRL in primates [4, 6, 16]. Moreover, chronic PRL treatment caused a notable hypertrophy of zona glomerulosa cells of the rat adrenal cortex [13]. PRL was also involved in the regulation of adrenal steroidogenesis in pigs. In a previous paper PRL administration increased plasma level of cortisol in the same sows [7]. However, in available literature there are no data pertaining to the effect of PRL on morphometric parameters of adrenal cortex in the pig.

Therefore the aim of this study was to examine the effect of experimental hyperprolactinemia on the stereologic parameters of adrenal cortex in sows during the early luteal phase of the oestrous cycle.

MATERIAL AND METHODS

Porcine PRL purified by Dr K Kochman (Institute of Animal Physiology and Nutrition, Jabłonna, Poland) was used for infusions [9, 10]. This preparation of porcine prolactin was free of porcine GH, ACTH, LH and FSH as tested by polyacrylamide gel electrophoresis. All other chemicals were purchased from Sigma Chemical Co., St Louis, MO.

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Investigations were conducted following institutional guidelines and the protocol was approved by the Animal Investigation Committee. The study was performed on 10 multiparous crossbred sows. On the 13th day of the oestrous cycle jugular veins of the animals were cannulated. Cannulas were used for administration (48 h in 2 h intervals) of PRL (0.3 mg) or saline (control sows). Injections of PRL (or soline) began 4–20 h after the preovulatory LH surge. Occurrence of the preovulatory LH surge (the highest concentrations of LH) was estimated by monitoring of vaginal mucus electrical resistance as described earlier [3]. At the end of the administration period sows were slaughtered by electrical shock. Slaughter procedure was performed in such a manner as to avoid the occurrence of stress-related changes. Adrenals were immediately dissected. One adrenal gland of each sow was designated for histological examination. Adrenals freed of adhering fat were fixed in Bouin's solution and embedded in paraffin. Subsequently, serially cut sections (5–6 μm) were stained with haematoxylin and eosin for stereology as well as the Masson method for microscopic examination. Additionally, the green filter (GIF) was used for histological observation.

Stereologic studies were performed in two stages. In stage I, using a magnification of 100 × and a square lattice test system of type A, the volume of the zona glomerulosa (ZG), zona fasciculata (ZF), zona reticularis (ZR), and adrenal medulla (M) were estimated by differential point counting, as described previously [12, 19]. The specific weight of the adrenal gland was assumed to be 1.039 mg/mm^3. In stage II, the size and number of adrenocortical cells were estimated on a screen at 3000 ×, using the multipurpose test system M42. Stereologic results were expressed per one adrenal gland.

Results were expressed as means ± SEM. Morphometric data (n = 5/group) were log transformed and then submitted to the T-test for independent variables (Statistica, StatSoft Inc., Tulsa, OK., USA).

Table 1. The morphometric parameters of the cortex of the left adrenal gland of saline and prolactin-treated sows during early luteal phase of the oestrous cycle. Results expressed as means ± SEM

	Saline	Prolactin
Adrenal gland weight [mg]	6456 ± 231	5994 ± 756
Volume [mm^3]		
the entire gland	6213.7 ± 222.2	5769.0 ± 727.5
zona glomerulosa	567.5 ± 36.4	489.1 ± 61.5
zona fasciculata	2614.8 ± 61.6	2425.6 ± 421.4
zona reticularis	1219.1 ± 217.4	1350.1 ± 433.0
medulla	1255.4 ± 154.5	992.8 ± 113.8
Relative volume of the gland (%)		
zona glomerulosa	9.15 ± 0.54	8.40 ± 0.35
zona fasciculata	42.25 ± 1.47	42.86 ± 5.76
zona reticularis	19.44 ± 3.03	22.36 ± 5.72
medulla	20.07 ± 2.09	17.46 ± 1.17
Average cell volume [mm^3]		
zona glomerulosa	966 ± 81	976 ± 68
zona fasciculata	1682 ± 110	1957 ± 304
zona reticularis	908 ± 71	923 ± 78
Average nuclear volume [mm^3]		
zona glomerulosa	162 ± 16	167 ± 10
zona fasciculata	149 ± 14	176 ± 25
zona reticularis	102 ± 13	114 ± 12
Distribution of adrenocortical cells (%)		
zona glomerulosa	16.75 ± 0.96	16.64 ± 2.20
zona fasciculata	46.75 ± 5.40	44.47 ± 8.01
zona reticularis	36.51 ± 5.20	38.89 ± 7.53
Number of adrenocortical cells (1×10^6)		
zona glomerulosa	558.5 ± 71.5	469.1 ± 41.5
zona fasciculata	1502.4 ± 90.5	1391.6 ± 398.8
zona reticularis	1303.7 ± 341.8	1174.0 ± 288.9
total	3364.6 ± 359.1	3034.7 ± 505.4

RESULTS

Analysis of the morphometric parameters

Results of histological examination are presented in Table 1. Statistical analysis of the data did not reveal any significant differences between control and PRL--treated sows. Hyperprolactinemia induced by 2-day administration of exogenous PRL did not affect the volume of particular cortical zones, the number and the volume of adrenocortical cells as well as the average volume of their cell nuclei.

Microscopic observation

In all examined sows the adrenal gland had three cortical zones clearly identifiable by arrangement and stainability of cells: zona glomerulosa, zona fasciculata and zona reticularis (Fig. 1). The thickness of the cortex and all the zones remained fairly constant in adrenals from both normal sows and sows after PRL administration.

Subcapsularly lying the zona glomerulosa (Fig. 1A) was created by oval or cuboidal epithelial cells

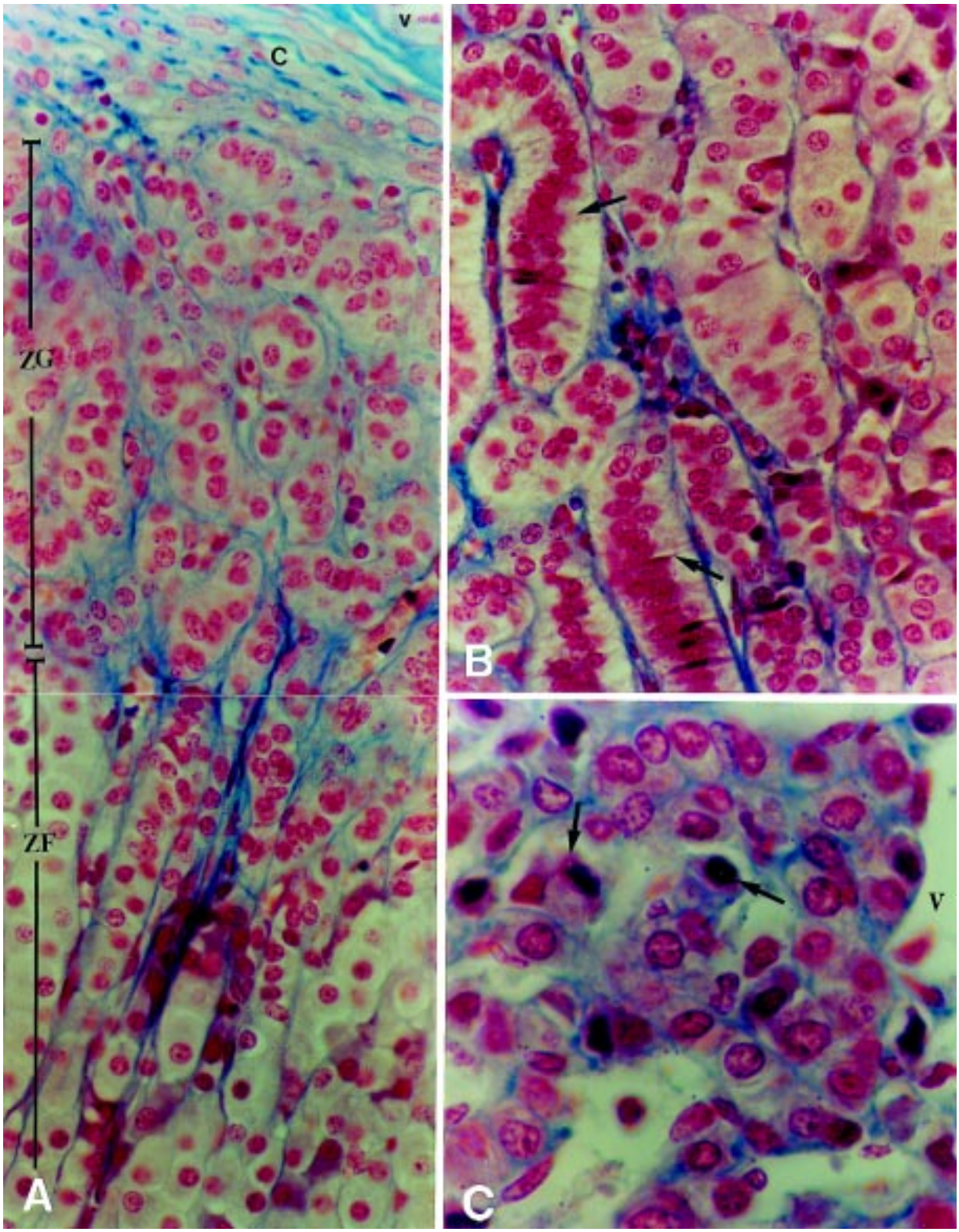

Figure 1. Cross-sections of the porcine adrenal cortex after staining acc. To Masson's method: **A.** Adrenal gland capsule (C) with the blood vessel (V), zona glomerulosa (ZG) and zona fasciculata (ZF) with cuboidal cells (× 750); **B.** Zona fasciculata with bands of long cylindrical cells (arrows) (× 750); **C.** Zona reticularis — epithelial cells arrangement longways the blood vessels of tiny walls (V) and visible numerous small cells with dark nucleus and cytoplasm (arrows) (× 1000).

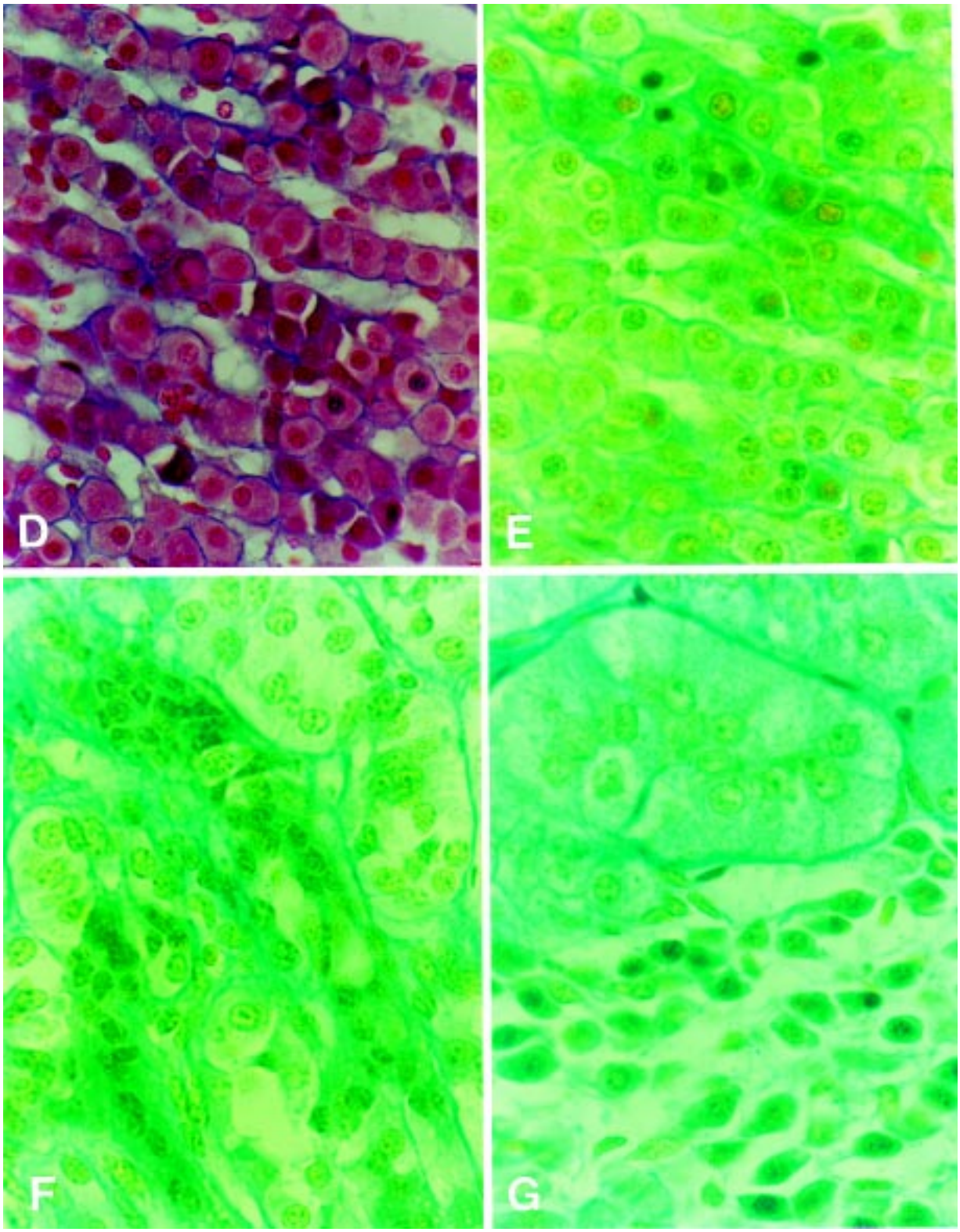

Figure 1. Adrenal cortex cross-section after staining acc. To Masson's method (D) and Masson/GIF (green filter). **D** and **E.** Zona fasciculata; **F.** Fragments of zona fasciculata and zona reticularis; **G.** The conglomeration of loose cells near the zona glomerulosa (D–G × 750).

collected in cluster (glomerules) surrounded by delicate connective tissue fibres. Between the zona glomerulosa and zona fasciculata of frequent occurrence were blood vessels and free-lying various cells (Fig. 1G). The zona fasciculata, constituting almost 2/3 of the cortical cross-sections, possessed long

cords, mainly with cuboidal cells of light frontier cytoplasm and round nucleus (Fig. 1A). However, some cords were formed by narrow cylindrical cells (Fig. 1B) or cords consisted of numerous cells with pycnotic nucleus and shrink cytoplasm (Fig. 1D, E). In some areas of the zona fasciculata the compaction of the connective tissue was visible (Fig. 1F). The zona reticularis was formed by epithelial cells lying longways of thinlywall blood vessels. There, various types of cells were visible (Fig. 1C).

DISCUSSION

The present study for the first time gives a detailed stereologic description of the adrenal cortex in the pig. Only Baba [2] reported planimetric data on the zonation of the pig adrenal cortex — in his study the area of cortex occupied by the glomerulosa and fasciculata — reticularis zones was 11.9 and 88.0%, respectively. Like in other mammalian species, in the pig adrenal cortex the best developed is the fasciculata zone, the cells of which are the largest and the most numerous (c. 47%) among the parenchymal cells of the cortex. The zona reticularis cells comprise about 37% of all parenchymal cells of the gland while glomerulosa cells only c. 17%. Thus, the zonal and cellular composition of the porcine adrenal cortex resembles that of some other mammalian species [for review see 11].

As demonstrated in the present study, hyperprolactinemia induced by two-day PRL administration changed neither the cellular composition nor the parenchymal cell size of the porcine adrenal cortex. In the rat, short-term PRL-treatment did not alter zona glomerulosa morphology or plasma aldosterone concentration but within 15 days PRL administration resulted in a hypertrophy of the zona glomerulosa cells and elevation of blood aldosterone level [13]. The lack of changes in the structure of the pig adrenal cortex observed in the present experiment is probably connected with a too short PRL stimulation of the adrenal glands. As demonstrated in the previous paper, two-day PRL administration increased both plasma cortisol concentration and adrenal content of cortisol in the some sows used for present study as well as PRL-stimulated cortisol release by suspension of porcine adrenocortical cells [7]. Thus, the stimulatory effect of PRL on secretion and plasma concentration of cortisol was not accompanied by quantitative morphological alternations in the adrenal cortex.

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