

Immunohistological Study of LH-Immunoreactive Cells in the Porcine Anterior Pituitary

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ABSTRACT. Anterior pituitary glands were removed from 18 sows at different stages of their estrous cycle to immunohistologically study the relationship between the stage of the estrous cycle and state of the luteinizing hormone (LH)-immunoreactive cells. The LH-immunoreactive cells were grouped into three types according to the amount of LH-immunoreactive granules stored in the cytoplasm (types A, B, and C). The number of LH-immunoreactive cells fluctuated along with the stage of the estrous cycle. In the 1st and the 6th stages, numerous LH-immunoreactive cells were observed (398 ± 39 , 391 ± 60 cells/ 10^{-2} mm², respectively). In the 2nd stage, only a few cells were observed (6 cells/ 10^{-2} mm²) and from the 3rd to the 5th stages, their number gradually increased.—**KEY WORDS:** anterior pituitary gland, estrous cycle, LH-immunoreactive cell, swine.

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In porcine reproductive disturbance, the frequency of ovary disease is high. Various factors cause ovary disease to develop. An abnormal level of luteinizing hormone (LH), which plays a major role in ovulation and luteinization, is an important factor. Levels of LH in both blood and the pituitary gland have been determined in sows during different stages of their estrous cycle, during pregnancy and lactation [2, 6, 7, 10]. However, the relationship between LH content in the pituitary gland and reproductive disturbance is unclear. Recently, numerous immunocytochemical studies, both at the light- and the electron-microscopic levels, have helped to identify the cells producing this hormone in the fetal porcine pituitary gland but not in the mature porcine one [4, 5]. It is possible that analysis of the distribution of LH-immunoreactive cells in the pituitary gland provides us with a key to the interpretation of reproductive disturbance.

The purpose of this investigation was to study the relationship between the different stages of the estrous cycle and character of LH-immunoreactive cells in sows.

MATERIALS AND METHODS

Pituitary glands: Pituitary glands were collected from slaughtered Large White sows, each weighting over 150 kg, at different stages of the estrous cycle as classified by Tsumura *et al.* [12]. The estrous cycle of the sow was divided into six stages according to macroscopic findings in the ovaries. The six stages were as follows. The first stage: follicle (7–12 mm in diameter) stage; the 2nd stage: ovulatory stage; the 3rd stage: reddish body (functional corpus luteum or early regressing corpus luteum) stage; the 4th stage: yellow body (regressing corpus luteum) stage; the 5th stage: white body or small follicle (less than 4 mm in diameter) stage and the 6th stage: medium follicle (4–6 mm in diameter) stage. The reproductive organs of each animal were examined macroscopically, especially the state of the endometrium and cervix.

Immunohistological examination: The pituitary glands

were sectioned at the central sagittal plane and immediately fixed in Periodate-Lysine-Paraformaldehyde (PLP) fixative or Helly's fluid. The fixed ovaries were subsequently dehydrated and embedded in paraffin, and sagittal serial sections, 3 to 4 μ m thick, were made.

The sections were deparaffinized, hydrated, and the endogenous peroxidase activity was eliminated by exposure to 2% periodic acid for 10 min. To prevent nonspecific binding of anti-rabbit IgG goat serum, the sections were incubated for 10 min in diluted normal goat serum (1:100). For immunohistochemical determination, the sections were treated first with anti-serum to porcine LH (Specific for porcine LH β , cross reaction for porcine TSH and FSH were 5.7% and 1.7%, respectively) produced in a rabbit, diluted 1:400 (UCB-bioproductions S.A.) for 30 min at room temperature. They were then treated with peroxidase conjugated anti-rabbit IgG goat serum diluted to 1:100 (Miles Laboratories, Inc.) for 30 min at room temperature, colored by a diaminobenzidine reaction for 10 min and counterstained with methylgreen (pH. 4.0) for 5 min at room temperature. Control sections were incubated in non-immune rabbit serum instead of anti-LH serum.

To examine the frequency of the LH-immunoreactive cells in the anterior pituitary region except for the pars tuberalis, the mean number of cells per 10^{-2} mm² was calculated with an ocular plotting micrometer.

RESULTS

Macroscopic findings in reproductive organs: The macroscopic findings in the ovaries, uterus and cervix of each sow are summarized in Table 1. No abnormal findings were observed in these organs. All sows in the 1st stage revealed cervical rigidity.

Classification of LH-immunoreactive cells: The LH-immunoreactive cells were grouped into three types according to the amount of LH-immunoreactive granules stored in the cytoplasm, i.e., 1) type A: having a few LH-immunoreactive granules (Fig. 1A); 2) type B: having

Table 1. Macroscopic findings in reproductive organs

Sow No.	Number of			Number of			Endometritis or pyometra	Rigidity of cervix ^{h)}	Estrous stage ⁱ⁾	
	HF ^{a)}	LF ^{b)}	MF ^{c)}	SF ^{d)}	RB ^{e)}	YB ^{f)}	WB ^{g)}			
1		15	<10	>15			>10	— ^{j)}	+++ ^{m)}	I
2		12	<10	>15			>10	—	+++	
3		12	<10	>15			>10	—	+++	
4		10	<10	>15			>10	—	+++	
5		10	<10	>15			>10	—	++ ^{l)}	
6	4	10					>10	—	—	II
7			<15	>10	13		>10	—	—	III
8			<15	>10	12		>10	—	—	
9			<15	>10	14		>10	—	—	
10			<15	>10	13		>10	—	—	
11			<5	>10		10	>10	—	—	IV
12				>20			>10	—	—	V
13				>20			>10	—	—	
14			20	>10			>10	—	+ ^{k)}	VI
15			20	>10			>10	—	+	
16			20	>10			>10	—	+	
17			20	>10			>10	—	++	
18			20	>10			>10	—	++	

a) Hemorrhagic follicle after ovulation. b) Large follicle (7–12 mm in diameter). c) Medium follicle (4–6 mm in diameter). d) Small follicle (less than 4 mm in diameter). e) Reddish body. f) Yellowish body. g) White body. h) Classification was based on the work of Meredith [9]. i) Classification was based on the work of Tsumura, I., *et al.* [12]. j) —: No detection. k) +: Slight. l) ++: Moderate. m) +++: Severe.

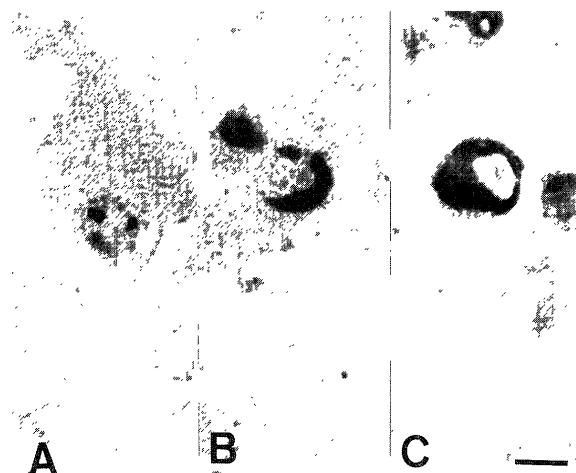


Fig. 1. Types of LH-immunoreactive cells. A: type A, having a few LH-immunoreactive granules in the cytoplasm, B: type B, having a moderate number of LH-immunoreactive cells, C: type C, having a large number of LH-immunoreactive granules. bar=10 μ m.

a moderate number of LH-immunoreactive granules (Fig. 1B); and 3) type C: having the cytoplasm filled with LH-immunoreactive granules (Fig. 1C).

Relationship between the stage of the estrous cycle and the number of LH-immunoreactive cells: The number of LH-immunoreactive cells fluctuated along with the stages of the estrous cycle. The LH-immunoreactive cells were distributed uniformly throughout the anterior pituitary

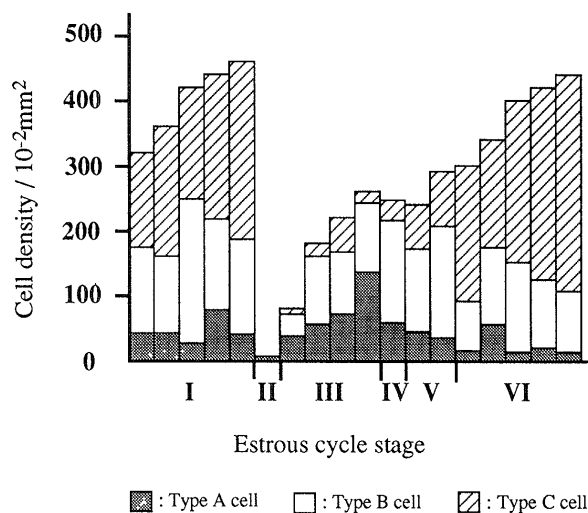


Fig. 2. Cell density of LH-immunoreactive cells in the anterior pituitary gland of each sow. Note that the number of the LH-immunoreactive cells changes with the stage of the estrous cycle.

except for the pars tuberalis. The frequency of LH-immunoreactive cells in the anterior pituitary is summarized and illustrated in Fig. 2. In the 1st and the 6th stages, numerous LH-immunoreactive cells were observed (Fig. 3). The numbers of these cells in the two stages were 398 ± 39 cells/ 10^{-2} mm² and 391 ± 60 cells/ 10^{-2} mm², respectively. In the 2nd stage, however, a much smaller number

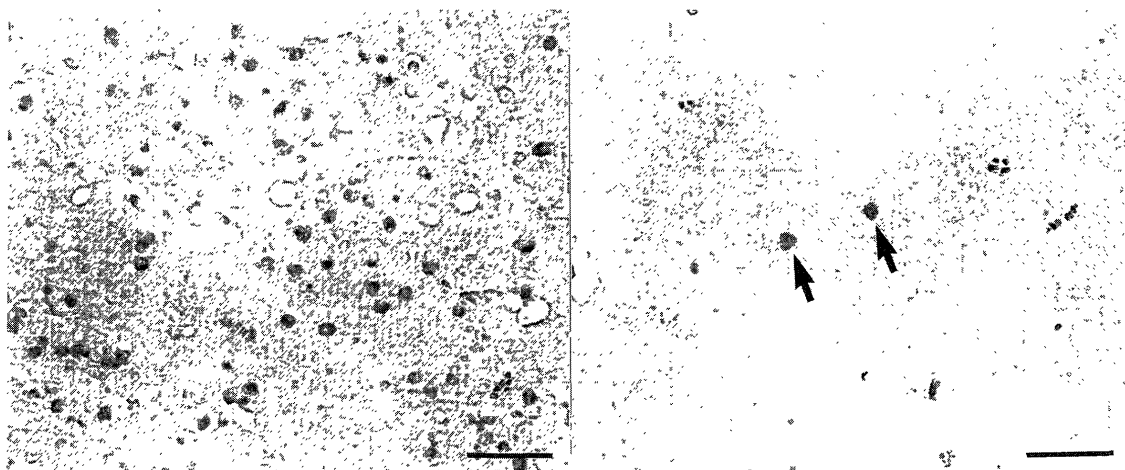


Fig. 3. An anterior pituitary gland from a sow in the 6th stage. Notice numerous LH-immunoreactive cells. bar=50 μ m.

Fig. 4. An anterior pituitary gland from a sow in the 2nd stage. A few LH-immunoreactive cells (arrows) are shown. bar=50 μ m.

of LH-immunoreactive cells were observed (6 cells/ 10^{-2} mm²) (Fig. 4). From the 3rd stage to the 5th stage, the number of these cells gradually increased.

The number of type A cell increased in the 2nd and the 3rd stages and that of type B in the 2nd to the 4th stage. Subsequently, both types of cells gradually decreased in the 5th and the 6th stages. On the other hand, the number of type C cells gradually increased in the 2nd to the 5th stage and continually increased, this time rapidly, in the 5th stage and 6th stages. In the 1st stage, the percentage of cells of each type in distribution was similar to that in the 6th stage.

DISCUSSION

The present study indicated that the number of LH-immunoreactive cells varied along with the stage of the estrous cycle. Fewer LH-immunoreactive cells were present in the 2nd stage, suggesting that pituitary LH had already been released into the blood. The change in the number of these cells was in agreement with that in the LH content of the pituitary which was measured by radioimmunoassay [10]. The results of our study suggested that each stage of the estrous cycle in the sow can be determined by measuring the number of LH-immunoreactive cells in the anterior pituitary.

Our study showed that type A cells have stored much less LH than type C cells which always have a large quantity of LH-immunoreactive granules, indicating a big store of LH. On the basis of changes in the number of each cell type alongside with the stages in the estrous cycle, it is possible to assume that type A cells become type B ones and then type C.

It is generally accepted that gonadotropic cells in the pituitaries of mammalian species synthesize both LH and FSH [3, 8]. Dacheux reported that, of the total gonadotropic cells the percentages of cells which stored only LH and those which stored both LH and FSH were 8–19% and

67–79%, respectively [3]. However, he did not indicate the fluctuation in the population of LH-containing cells during the estrous cycle. The present study showed that the population of LH-immunoreactive cells varied along with the stage of the estrous cycle. In our preliminary study, on the other hand, the detection of FSH-immunoreactive cells had been attempted with anti-serum to bovine FSH, but no positive findings were obtained. In order to clarify the relationship between the fluctuating ratio of LH-containing cells to FSH-containing ones, it will be necessary to detect FSH-immunoreactive cells with anti-serum to porcine FSH.

In the present study, the number of LH-immunoreactive cells in the 1st stage was close to that in the 6th stage. Considering the period of the LH surge, the number of LH-immunoreactive cells in the 1st stage did not correspond with changes in the plasma LH level. This unexpected finding led us to consider that in the sows in the 1st stage used in this study, either estrus had not started or the LH surge had not occurred. In sows, standing behavior and cervical rigidity are well known signs of estrous onset [9]. In this study, rigidity was clearly observed in all cases in the 1st stage. Other reports suggested a relationship between follicle diameter and the estrous cycle [1, 11], which agrees with our finding that many large follicles over 7 mm in diameter existed in all the ovaries in the 1st stage. Therefore it is unlikely that these sows were in the previous stage before estrus, but rather that estrus had already started without a subsequent LH surge from the pituitary gland.

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REFERENCES

1. Anderson, L. L. 1980. pp. 369–372. *Reproduction in Farm Animals*, 4th ed. (Hafez, E.S.E. ed.), Les & Febiger, Philadelphia.
2. Chakraborty, P. K., Reeves, J. J., Arimura, A., and Schally, A. V. 1973. Serum LH levels in prepubertal female pig chronically treated with synthetic luteinizing hormone-releasing hormone/follicle-stimulating hormone-releasing hormone (LH-RH/FSH-RH). *Endocrinology* 92: 55–61.
3. Dacheux, F. 1981. Proportions of FSH/LH cells, LH cells and FSH cells in the porcine anterior pituitary. *IRCS Med. Sci.* 9: 952–953.
4. Dacheux, F. 1984a. Differentiation of cells producing polypeptide hormone (ACTH, MSH, LPH, α - and β -endorphin, GH and PRL) in the fetal porcine anterior pituitary. *Cell Tissue Res.* 235: 615–621.
5. Dacheux, F. 1984b. Functional differentiation of the anterior pituitary cells in the fetal pig. An ultrastructural immunocytochemical study. *Cell Tissue Res.* 235: 623–633.
6. Day, B. N., Anderson, L. L., Naze, L. N., and Melampy, R. M. 1959. Gonadotrophic and lactogenic hormone potencies of gilt pituitaries during the estrous cycle and pregnancy. *J. Anim. Sci.* 18: 675–682.
7. Hollandberck, R., Baker, B. J., Norton, H. W., and Nalbandov, A. V. 1956. Gonadotrophic hormone content of swine pituitary glands in relation to age. *J. Anim. Sci.* 15: 418–427.
8. Liu, Y. C., Kato, Y., Inoue, K., Tanaka, S., and Kurosumi, K. 1988. Colocalization of LH β and FSH β mRNAs in the porcine anterior pituitary by *in situ* hybridization with biotinylated probes. *Biochem. Biophys. Res. Commun.* 154: 80–84.
9. Meredith, M. J. 1977. Clinical examination of the ovaries and cervix of the sow. *Vet. Rec.* 101: 70–74.
10. Niswender, G. D., Reichert, L. E. J., and Zimmerman, D. R. 1970. Radioimmunoassay of serum levels of luteinizing hormone throughout the estrous cycle in pigs. *Endocrinology* 87: 576–580.
11. Schilling, E. 1974. Stages of ovarian function in the sow. *Vet. Med. Rev.* 1: 59–63.
12. Tsumura, I., Sasaki, H., Minami, S., Hirayama, M., Kurosaka, S., and Nonami, K. 1981. Studies on the rigidity of the cervix of sows. *Jpn. J. Anim. Reprod.* 27: 65–73.