

TRANSITION METAL CONTENTS IN THE OVARIES OF SOWS DURING ESTROUS CYCLE

著者	ITABASHI Hiroko, ISHIDA Kazuo
journal or publication title	Tohoku journal of agricultural research
volume	8
number	1
page range	11-16
year	1957-07-31
URL	http://hdl.handle.net/10097/29221

TRANSITION METAL CONTENTS IN THE OVARIES OF SOWS DURING ESTROUS CYCLE

By

Hiroko ITABASHI and Kazuo ISHIDA

*Department of Animal Husbandry, Faculty of Agriculture,
Tohoku University, Sendai, Japan*

(Received June 6, 1957)

Introduction

Since the work of Fevold, Hisaw and Greep (1) who discovered that copper salts would induce ovulation in the rabbit, it has been known that many biological phenomena are affected by the transition metals. McElroy and Glass (2) reported that copper deficiency in food was found in some areas of New Zealand; cows and sheep undernourished and diminished in their abilities of reproduction. Fay *et al.* (3) observed the increase of copper contents in the plasma of pregnant women. Axtrup (4) and Morrison and Nash (5) found that human fetal livers contained five to ten times as much copper as adult livers.

It has been known that some metals are the active centers of prosthetic groups of some enzymes and operate greatly as the activators in the biological systems. However, the relation of the sex hormones to the metals and that of the metals to the estrous cycle are not known.

This investigation was undertaken in order to clear the relation of transition metals to the estrous cycle by the determination of the metals in the ovaries of sows.

Materials and Methods

I. Preparation.

The ovaries of Yorkshire sows were obtained at the Sendai slaughter house during from the middle of November to the end of December 1954. They were divided into 5 groups by the Corner's classification (6), washed clean with redistilled water, put into a crucible and weighed. After drying at 105°C the ovaries were heated to give white ash. The ash was solved by pouring dilute hydrochloric acid (1:1) into the crucible, and then transferring into a messflask. An aliquot of the solution was estimated to be used for this

study.

II. Methods.

1. *Qualitative method.*

Qualitative determination was carried out by the paper chromatographic method of Kikkawa and Ogita (7) as follows.

The solvent stems used were composed of *n*-BtOH-AcOH-H₂O (4:1:5) and of Acetone-*n*-BtOH-HCl (10:4:2) respectively. To develop the specific colours for copper, cobalt and nickel, a sheet of paper treated with *n*-BtOH-AcOH-H₂O was exposed to ammonia gas after spraying with 0.1~0.5 per cent rubeanic acid alcohol solution. To develop the colour for molybdenum, the similar treatment was employed on another sheet after spraying with 1~5 per cent pyrocathocol solution, and to develop that for iron the third sheet which was treated with Acetone-*n*-BtOH-HCl was exposed to dilute hydrochloric acid after spraying with 1 per cent potassium ferrocyanide solution.

2. *Quantitative method.*

For the quantitative determination of the copper, nickel and iron, the systematic method which had been originally introduced by Sandell (8) and modified by Kikkawa and Ogita (7) was employed as follows.

a) Quantitative isolation of copper and nickel by dithizone, and estimation of them.

An aliquot of the sample was poured into a small separatory funnel of Squibb type and 2 ml of 20 per cent ammonium citrate solution was added. It was then adjusted to pH 8.5~9.0 with 5 N ammonia water. After adding 5 ml of 0.03 per cent dithizone carbon tetrachloride solution, these metals were transferred after vigorous shaking into the solution forming dithizone complexes which gave a reddish violet colour. This operation was repeated with a new dithizone solution until it remained green. Further complete nickel extraction was carried out at pH 9.8 as follows: After adding 1 N hydrochloric acid to make the solution 0.05 N concentration, the solution was shaken to extract the nickel from their dithizonate, leaving copper dithizonate.

To isolate the copper from the dithizonate, 20 ml of 0.05 N hydrochloric acid solution was added, and the copper was transferred into the solution when the dithizonate was decomposed by addition of some drops of bromine water and shaken vigorously. The carbon tetrachloride solution was discarded. 1 ml of 20 per cent ammonium citrate was added to the aqueous solution. After adjusting it to pH 9.0~9.2 with ammonia water 1 ml of 0.1 per cent diethyldithiocarbamate was added as the developing reagent: after adding 10

ml of isoamylalcohol, the coloured copper was extracted into it by vigorous shaking. When left alone for a moderate time the solution was estimated with filter S-42 by Hitachi photometer.

To the hydrochloric acid solution containing nickel, 1 *ml* of 20 per cent ammonium citrate was added to adjust to pH 8.5~9.0. After the addition of 2 *ml* of 1 per cent dimethylglyoxim and 5 *ml* of chloroform, the solution was shaken vigorously. The nickel in the chloroform layer was treated as follows: 0.5 *N* hydrochloric acid was added to the chloroform layer and then 10 drops of bromine water and concentrated ammonia water. After vigorous shaking some more ammonia water and 1 *ml* of 1 per cent dimethylglyoxim were added and then the solution was estimated photometrically with green filter S-54.

b) Quantitative estimation of iron.

An aliquot of the sample solution was poured into a messflask. One milliliter of 10 per cent ammonium citrate was added and the pH was adjusted to 5.5 with dilute ammonia water or with 5 *M* sodium acetate solution. One milliliter of 1 per cent quinone solution and 2 *ml* of 0.25 per cent *o*-phenanthroline were added to develop the colour. The solution was diluted to an adequate concentration and estimated with the filter S-53 by the photometer.

Results

1. Result of the qualitative analysis.

Qualitative analysis of metals was made by the method as already mentioned with the following results.

Intense colour reaction of iron (blue) and of copper (dark green) and weak colour reaction of nickel (blue-violet) were found. No reaction of molybdenum and cobalt was found.

2. Result of the quantitative analysis.

Quantitative analysis was made by the method as already stated and the metal contents in the ovaries of sows are given in Table 1. In the same table are also given the general aspects of the ovaries used.

As shown in Table 1, copper contents were rich in the immature ovaries which usually lack corpora lutea. With mature sows, the copper contents were poor in the diestrus, being estimated to be 3.8 γ /g on the average. They increased with the stages of estrous cycle and reached the maximum at the estrus, being estimated to be 11.0 γ /g on the average, which then began to decrease, and finally reached the minimum in the diestrus.

Nickel contents were slight. The amount varied with individuals, ranging

Table 1. Metal contents in the ovaries of sows

stage	immature					proestrus					estrus					
	metals	Cu	Ni	Fe	aspects of ovaries		Cu	Ni	Fe	aspects of ovaries		Cu	Ni	Fe	aspects of ovaries	
					W.	F.				W.	F.				W.	F.
	γ/g				g	mm										
	12.2	2.6	17.9	5.3	5	6.6	0.03	36.4	11.0	8	9.8	3.5	29.6	7.3	11	
	6.2	—	21.7	6.9	4	6.8	1.4	15.8	6.0	6	9.6	0.1	2.2	4.2	11	
	8.5	2.1	14.3	9.1	5	6.3	1.1	9.1	5.6	8	8.6	1.7	26.5	6.8	9	
	10.7	1.7	33.8	7.1	7	10.3	1.0	—	25.4	13	11.4	1.9	30.5	6.0	10	
	14.5	1.2	21.6	5.6	5						15.5	1.0	—	1.0	13	
mean	10.4	1.9	21.9			7.5	0.9	20.4			11.0	1.6	22.2			

W — Weight
 F. — Follicle
 C. L. — Corpora Lutea

from 0.0 to 3.5 γ/g , but not in stages. Iron contents showed wide variations both in individuals and in stages, being estimated to be from 2.2 to 36.4 γ/g , due to the blood hemoglobin in the ovaries.

Discussion

Pedersen-Bjergaard (9) found that 175 M. U. estrogens were contained in the urine of women on the 10th day and 160 M. U. estrogens on the 24th day after the menstruation. Kika *et al.* (10) reported that the estrogens were rich in the urine of women; arranged according to the amount, the order was estrone, estradiol and estriol, and they reached their maximum on the 17th day after the menstruation when the ovulation occurred. These results show that the estrogen contents in the urine of women were rich on the 17th day after the menstruation and were poor on the 24th day after the menstruation.

In the present investigation, the copper contents in the ovaries of sows were at the maximum in the estrus and at the minimum in the diestrus. This variation during the estrous cycle of sows almost agreed with that of the estrogen contents during the menstrual cycle of women by Pedersen-Bjergaard and Kika *et al.*, showing the intimate relationship between the copper and the estrogens.

Summary

The results obtained in this investigation are summarized as follows:

at each stage of the estrous cycle.

mature											
metestrus						diestrus					
Cu	Ni	Fe	aspects of ovaries			Cu	Ni	Fe	aspects of ovaries		
			W.	F.	C. L.				W.	F.	C. L.
4.5	1.1	22.2	9.9	4	4	3.3	1.0	19.3	13.2	9	12
4.4	0.2	5.2	15.4	7	13	3.9	0.8	9.3	17.3	5	15
5.6	0.9	7.5	11.3	7	9	6.7	1.9	23.6	11.0	6	11
4.6	1.1	9.9	20.7	4	5	5.4	1.5	17.6	5.3	5	10
3.7	0.2	7.5	12.6	5	13	2.1	0.6	—	15.2	6	10
						3.1	0.7	14.4	13.9	4	12
						1.9	0.0	2.7	15.0	4	14
4.6	0.7	10.5				3.8	0.9	14.4			

(The largest follicle was measured.)

(The largest corpus luteum was measured.)

1. Intense colour reaction of iron (blue) and of copper (dark green) and weak colour reaction of nickel (blue-violet) were found by the paper chromatographic method. No reaction of molybdenum and cobalt was found.

2. Copper contents were rich in the ovaries of immature sows, being estimated at 10.4 γ/g on the average.

3. In the ovaries of mature sows, the copper contents were poor in the diestrus, being estimated to be 3.8 γ/g on the average. The copper contents increased with the stages of estrous cycle up to the estrus when they were estimated to be 11.0 γ/g . They began to decrease and reached the minimum in the diestrus.

4. Nickel contents were slight in every stage of the estrous cycle.

5. Iron contents showed variations both in individuals and in stages, due to the blood hemoglobin in the ovaries.

Acknowledgment

The authors express their hearty thanks to Prof. Dr. Kikkawa and Mr. Ogita for the chemical analysis of the metals, and to Prof. Dr. Y. Toryu for his kindness throughout the course of this work. Thanks are also due to the Sendai slaughter house for supplying the materials used.

References

- 1) Fevold, H. L., F. L. Hisaw and R. Greep : (1936). Amer. Jour. Physiol., **117**, 68.

- 2) McElroy, W. D. and B. Glass : (1950). Copper metabolism, p. 246. The Johns Hopkins Press, Baltimore.
- 3) Fay, J., C. E. Cartwright and M. M. Wintrobe : (1949). *Jour. Clin. Invest.*, **28**, 487.
- 4) Axtrup, S. : (1946). The blood copper in anaemias of children with special reference to premature cases, pp.146, A.-B. P. H. Lindstedts Univ.-Bokhandel, Haken Ohlssons Boktryckeri, Lund. (Cited from "McElroy, W.D. and B. Glass: Copper Metabolism, p. 278, 1950").
- 5) Morrison, D. B. and T. P. Nash : (1933). *Jour. Biol. Chem.*, **88**, 479.
- 6) Corner, G. W. : (1921). *Carnegie Inst. of Wash. Publ.*, No. 276, *Contr. to Embryol.*, **13**, 117.
- 7) Kikkawa, H. and Z. Ogita : (1965). *Seibutsu-Kagaku Saikin no Shimpo*, **1**, 292. (in Japanese).
- 8) Sandell, E. B. : (1950). *Colorimetric Determination of Traces of Metals*, Interscience Publishers Inc., New York.
- 9) Pedersen-Bjergaard, K. : (1936). *Zbl. Gynäk.*, **60**, 372.
- 10) Kika, K., G. Suzuki and S. Mori : (1955). *Nabumpitsu*, **2**, 175. (in Japanese).