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## Hydrochloric Acid Gastric Secretion in Rats at First Trimester.

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### ABSTRACT

The problem of early toxicosis is quiet current today. Nausea and vomiting in pregnant are the most common forms of complications during the early stages of pregnancy. Vomiting is a complex clinical syndrome, characterized by digestive disorders, leading to significant violations of organism condition. Changes in gastric basal and carbachol stimulated acid output (BAO, CAO) were studied in rats during pregnancy. CAO increased on the 3rd, 5th and 7th day of gestation compare to non-pregnant animals. On the contrary, on the 4th day of gestation CAO decreased. These results suggest that the gastric acid secretion varied during pregnancy. Obviously, such variances were associated with the increase of sex hormones and other endogenous substances concentration.

**Keywords:** estrous cycle, toxicosis, gestation, gastric acid output.

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## INTRODUCTION

The problem of early toxicosis is quiet current today. Toxicosis is a condition of pregnant women that occur due to development of whole fertilized egg or its separate elements. Central nervous system, vessels, metabolism disorders are the most common and distinct disturbance that characterize toxicosis. There are early toxicosis and late gestosis. The main difference between the states is in their clinical flow. Early toxicosis usually develops in first and ends at the beginning of second trimester. Gestosis appears in second and third trimesters. Nausea, vomiting, excessive salivation, gastroesophageal reflux [1–5], heartburn [6] are the common form of early toxicosis. Also there are rare forms such as dermatitis, tetany, osteomalacia, acute yellow atrophy of the liver, asthma in pregnant [7].

Nausea and vomiting in pregnant are the most common complications that occur during the early stages of pregnancy. Early toxicosis observes in 40-60% of mothers. Clinical manifestations of nausea and vomiting that require special treatment, observe in 15-18% of pregnant women, severe form - in 0.5% of women. Vomiting is a complex clinical syndrome, characterized by digestive disorders, which leads to significant violations: neuroendocrine regulation, dehydration, changes in metabolic and electrolyte balances, delay glucose absorption [6]. Starvation, dehydration, exhaustion and weight loss lead to water-salt (hypokalaemia), carbohydrate, fats and protein metabolism disturbance.

Changes in the organs transfer into the dystrophic processes due to dehydration, acceleration of catabolic reactions, and intoxication by oxidized products. At first the main functions of liver, such as protein synthetic, antitoxic, pigment and kidneys excretory function are disturbed. Further, degenerative changes in the central nervous system, lungs, heart are observed. Such complications affect on the overall health of pregnant women as well as on the pregnancy flow and fetal growth.

There are many theories that explain the development of early toxicosis, but among physicians and scientists there are no single point of view about its etiology. Some considers that the main mechanism of toxicosis is connected with the central nervous system (CNS) of women. According to these theories at the moment of fertilization the functioning of CNS changes, as the result CNS causes nausea, vomiting, increased salivation, changes in sapor (taste) and olfaction.

Other scientists suppose that toxicosis is the defense response of pregnant women organism. Also such changes may occur due to chronic diseases, malnutrition and nervous tension. Thus, the theories of early toxicosis development contradict to each other and not fully explain toxicosis emergence.

Investigation of gastric acid secretion changes during pregnancy are not completely: both increase [8,9] and decrease [10–13] of the hydrochloric acid output are proper for animals. Vice versa, in human there is a tendency to reduction of acid output [6] and, even, to a lack of hydrochloric acid [13]. Scientists showed that during first 30 weeks of pregnancy gastric acid output (GAO) was reduced, in another work - GAO reduced after the 15<sup>th</sup> week of pregnancy. Due to GAO decrease the relief of ulcer disease symptom is frequent [6,11]. It was shown that both gastric tonus and motility decreases during the pregnancy [6,14]. We assumed that high concentration of such hormones as estrogen, progesterone, human chorionic gonadotropin influence on gastric parietal cells and in such way suppress GAO, as follows early toxicosis develops [10,15–17]. The reduction of hydrochloric acid secretion leads to dysbiosis, putrefactive processes and suppression of proteolytic protein activation.

However, it is quite difficult to investigate the GAO of pregnant women, so we decided to carry out investigation on rats with the similar terms of pregnancy. Aim of work was to study the basal and stimulated acid output in non-pregnant rats and at early terms of pregnancy.

## MATERIALS AND METHODS

### Animals

The study was carried out on white nonlinear rats 4-5 months old and with weight 180-200 g in strict accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health and the general ethical principles of animal experiments, approved by the First National

Congress on Bioethics Ukraine (September 2001). The protocol was approved by the Committee on the Ethics of Animal Experiments of the Taras Shevchenko National University of Kyiv. Females were kept separately from males since 1.5 months age. The rats were kept in collective cages in controlled conditions of temperature ( $22\pm 3^{\circ}\text{C}$ ), light (12h light/dark cycle) and relative humidity ( $60\pm 5\%$ ). The animals were fed laboratory chow and tap water *ad libitum*.

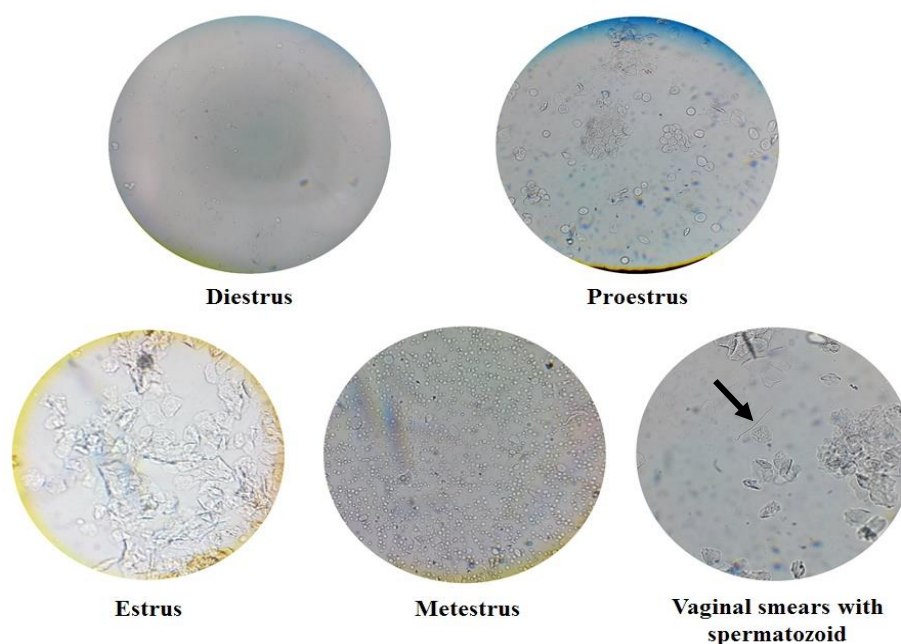
#### Determination of estrous cycle phase and dated of pregnancy.

To detect estrous cycle phases and to register first day of pregnancy the vaginal smears were used. The stage of the estrous cycle for each female rat was determined by histological examination of cells in vaginal smears, that were taken daily from 10:00 a.m. till 12:00 a.m. [18,19]. In the 60-70% of rats average duration of the estrous cycle is 4 to 5 days; therefore, the animals with irregular cycle were removed from the experiment. Each vaginal smear was taken using a new cotton swab dipped in distilled water. The unstained native smears were immediately studied under the microscope.

The following estrous cycle phases were distinguished: diestrus 1 (D1, also called metestrus), diestrus 2 (D2), proestrus (P), and estrus (E) [20]. The phases of the estrous cycle were determined according to cytological profile of vaginal smears. Metestrus was characterized by the three cell types: unnuclated cornified cells and epithelial cells, leucocytes. At the end of the stage leucocytes dominated, the mucus was appearing and keratinized cells were vanishing. Diestrus smear contained leucocytes with few larger round cells, single epithelial cells and considerable amount of mucus. Proestrus smear consisted of round or polygonal nucleated epithelial cells with granular cytoplasm and big nucleus. These cells were located separately or in small groups. Estrus was characterized by presence of large amount of keratinized cells in smear, leucocytes and epithelial cells were completely absent. At the end of estrus keratinized cells formed clusters [18,19,21]. Using this method, we were able to classify the stages of the estrous cycle in each rat after observation of 2-3 cycles.

For mating healthy females and males were selected. Only young females were observed, because with age estrous cycle phase become uneven and irregular. To make mating successful only females in proestrous phase were used [22]. Breeding rats were housed together (in ratios of 1:1) overnight until the start of light cycle the next day. In the morning after mating female was removed from cage and observation of spermatozoids in vaginal smears were conducted to prove mating. Female has been engaged in the subsequent experiment in case of spermatozoids presence. The day on which spermatozoids were found in a vaginal smear was called the first day of gestation [15–17,22–25].

Picture 1: Estrous cycle phases that were determined according to cytological profile of vaginal smears.



Gastric acid output were studied on 3rd, 4<sup>th</sup>, 5<sup>th</sup> and 7<sup>th</sup> day of gestation, which corresponds to the 5<sup>th</sup>, 8<sup>th</sup> and 12<sup>th</sup> week of pregnancy in women [26,27]. Because of small pregnancy term we confirmed the presence of gestation by uninterrupted analysis of smears and, also, by uterus precise examination. After cutting the uterine horns were examined to confirm the presence of larger blood supply and increase in size [28–31], and the presence of corpora lutea. At the 7<sup>th</sup> days of pregnancy the embryos were observed.

**Methods of gastric acid output study**

In 24 hours before the experiment animals had no food, but they had free access to water. Carbachol stimulated gastric acid output (CAO) was investigated in acute experiments on 130 white female rats. The method of isolated stomach perfusion by Ghosh and Shild was used [32]. Rats were anaesthetized by urethane (Sigma Chemical Co, St. Louis, USA) at the dose 1.1 g/kg intraperitoneally [33]. After the experiments animals were sacrificed with lethal doses of urethane (3 g/kg) [34].

The quantity of hydrochloric acid in the perfusate was measured by back-titration method with ion meter. The quantity of 0.01 N NaOH that was used for titration of one 10-minute samples was equal to a debit of hydrochloric acid in this sample in back-titration units. To calculate basal acid output (BAO) that lasted 2 hours, all debits of 10-minute samples were summed. After the first 2 hours the rats were injected with carbachol (0.01 mg/kg) i/p. CAO was also measured for 2 hours.

All obtained data were subjected to statistical analysis using software package “Statistics, 8.0”. Shapiro-Wilks criterion was used for the analysis of data distribution type. As the data were normal distributed independent samples were compared by Student’s t-test. The difference between two means was considered to be statistically significant when *P* was less than 0.05 [35]. We calculated the mean value (M) and standard deviation (SD).

**RESULTS**

It was found that BAO of pregnant and non-pregnant (control) rats had no significant difference within all days of study (table 1). We observed only a tendency of the BAO reduction.

**Table. 1. Basal and carbachol stimulated acid output (M±SD)**

	Non-pregnant rats	3 <sup>rd</sup> day of pregnancy	4 <sup>th</sup> day of pregnancy	5 <sup>th</sup> day of pregnancy	7 <sup>th</sup> day of pregnancy
Basal acid output	2.42±0.75 n=11	1.34±0.45 n=11	1.30±0.39 n=11	1.29±0.42 n=11	1.41±0.44 n=11
Carbachol stimulated acid output	15.72±3.83 n=15	24.53±4.52* n=15 ↑by 56%	23.94±4.12* n=15 ↑by 52%	4.48±2.78* n=15 ↓by 71,5%	24.41±4.34* n=15 ↑by 55%

\* - *p* < 0.05 compared with the control; n – amount of rats;

**Table 2: Basal and carbachol stimulated acid output on 5th day of pregnancy (M±SD)**

	Non-pregnant rats	5 <sup>th</sup> day of pregnancy	Effect (%)
Basal acid output	2.42±0.75 n=11	1.29±0.42 n=11	<i>p</i> >0.05
Carbachol stimulated acid output	15.72±3.83 n=15	4.48±2.78** n=15	71.5% <i>p</i> <0.01
		2.54±1.21*** n=9	84% <i>p</i> <0.001
		7.58±1.02** n=6	51% <i>p</i> <0.01

\* - *p* < 0.05; \*\* - *p* < 0.01; \*\*\* - *p* < 0.001 compared with the control; n – number of experiments

CAO of pregnant rats strongly differed from non-pregnant one in all periods of investigation. CAO in experimental rats on the 3rd, 4th and 7th days of pregnancy increased by 56% ( $p < 0.05$ ), 52% ( $p < 0.05$ ) and 55% ( $p < 0.05$ ) respectively compare to control.

However, on the 5th day CAO significant reduced by 71.5% ( $p < 0.01$ ). We analyzed the results of 5th day more precisely, it was found that in first group (60% of pregnant rats) carbachol didn't influenced on gastric acid secretion, and in second group (40% of pregnant rats) carbachol stimulated secretion. Moreover, in second group CAO was by 51% ( $p < 0.01$ ) lower than in the control rats. Our calculation showed that total CAO (all periods of pregnancy) of experimental rats decreased by 71.5% ( $p < 0.01$ ) in comparison with control (table 2).

## DISCUSSION

As it was mentioned above, the literature data about secretion of hydrochloric acid in the pregnant rats are contradictory. Some of authors describe the GAO reduction during the first 30 weeks of pregnancy [36], other points to the same beginning the 15th week of pregnancy [37]. For animals both increase [8,9] and decrease of hydrochloric acid secretion during the pregnancy are common [10–13].

We have obtained the data which showed that CAO varies during the first trimester. Our data was measured in dynamics, almost every day of the first trimester, so we can explain the contradiction about the secretion in literature taking into account that researchers studied the secretion in separate days of pregnancy. Obviously, such changes can be associated with the action of hormones: it is well known, that the work of body systems changes from the first days of pregnancy, and a large amount of hormones are secreted. Sex hormones secrete not evenly, and their concentration vary in the blood during the pregnancy. Also known, that sex hormones, such as estrogen, progesterone and human chorionic gonadotropin, are able to reduce the basal and stimulated acid output in both animals and humans, probably, inhibiting the secretory function of gastric parietal cells [10–12]. Even the lack of hydrochloric acid in gastric juice was observed during the first trimester [13]. In our experiments at the 5th day the secretion decreases due to the large amount of estrogen, namely estradiol. It is known from the literature, that a significant increase in estrogen secretion rate is observed on the 4th day of gestation. [15,38] It coincides with the implantation of the fetus. However, in the literature above mentioned state was described for rats that were kept in a constant temperature in the room (25 C) in which the lighting schedule is 14 hr light and 10 hr dark. Our experiments were conducted in winter, so the temperature was lower (20 C) and the daylight hours was shorter (10 hr). Therefore, the physiological time of estrogen secretion shifted and CAO reduced one day later.

The increase of CAO in our experiments on the 3rd, 4th and 7th days of pregnancy was due to the release of additional endogenous substances. Chen T.S. [8] showed that both pentagastrin and gastrin stimulated acid output were the biggest in first trimester of pregnancy, but not late. In rats histamine stimulated acid output was maximal only in the late pregnancy periods. Such situation can be explained by the facts that the level of serum histamine increases during pregnancy [39], and also ectopic gastrin is secreted by the placenta [37], and these substances enter in the gastric mucosa from the bloodstream [40]. The carbachol injection increases the secretion of hydrochloric acid due to the potentiation of the stimulants action [41]. The increased acidity of gastric juice causes several adverse effects such as heartburn, gastroesophageal reflux, dysbiosis, putrefactive processes, nausea and vomiting. Such changes in GAO lead to pregnancy complications and cause damage of mother's and child's health. Therefore, we assume that one of the causes of early toxicosis may be the suppression of gastric secretion because of influence of high concentration of hormones such as the estrogen, progesterone, human chorionic gonadotropin on the stomach parietal cells.

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