Balkan Med J 2011; 28: 1-9 • DOI: 10.5174/tutfd.2009.02492.1

Effects of Dimenhydrinate and Ondansetron Used in Pregnant Rats on Postnatal Morphometric Development

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ABSTRACT

Objective: To determine the effects of dimenhydrinate (DMH) and ondansetron, used as antiemetics during pregnancy, on the length of gestation, maternal weight gain during gestation and postnatal morphometric parameters of pups.

Material and Methods: Thirty female Wistar albino rats were used in the study. Three groups comprising 10 rats each were studied: Group 1: control group; Group 2: DMH group; and Group 3: ondansetron group. Rats were impregnated and administered 115 mg/kg/day intramuscular, DMH group, or 10 mg/kg/day intraperitoneal, ondansetron group. The parameters pertaining to the cranium, thorax and limbs were measured between newborn and adulthood.

Results: The increase in mean morphometric parameters during the postnatal period in the DMH group was less than the control group,whereas that in the ondansetron group exceeded the controls ($p<0.05$). Moreover, when data obtained between weeks 7 and 11 were analyzed for males and females separately, morphometric parameters increased at a slower rate in the DMH than the control group and morphometric parameters increased at a higher rate in the ondansetron than the control group in either sex ($p<0.05$).

Conclusion: DMH and ondansetron used during gestation affect weight gain during gestation, and morphometric development of pups during newborn and lactation periods and adulthood.

Key Words: Antiemetic, developmental anatomy, fetal development, gestation, rat

Received: *07.04.2009* ***Accepted:*** *19.05.2009*

Introduction

Nausea and vomiting are frequently observed symptoms of pregnancy, occurring in some 80% of all pregnancies (1-3). Nausea and vomiting occur between 4-16 weeks of gestation in the majority of cases, though it may persist throughout pregnancy in 20% of the cases (1). Nausea and vomiting continuing all day long, not responding to out-patient medical treatment, interfering with the nourishment and daily activities of the mother-to-be, causing poor general health state or weight loss is referred to as hyperemesis gravidarum (excessive vomiting of pregnant women). Hyperemesis gravidarum is observed in less than 1% of all pregnancies and requires hospitalization for further examination and treatment (1, 2, 4) since intrauterine growth retardation and fetal anomalies may develop in response to inadequate treatment or unresponsiveness to treatment with consequent weight loss, electrolyte imbalance, malnutrition and vitamin deficiency (5).

Although medical treatment is not given in all nausea and vomiting in pregnancy, it is administered in resistant hyperemesis gravidarum cases. DMH and ondansetron are two of the drugs that are used. Excessive nausea and vomiting may not be prevented by simple therapeutic measures (6-8). It has been reported that certain antiemetic drugs did not yield successful outcomes in hyperemesis gravidarum and that more potent drugs were required (1). Pre-clinical studies on the safety of ondansetron showed that it has a good safety profile (9). Ondansetron, an antiemetic, has been used and yielded successful results in hyperemesis gravidarum (6). In clinical practice, DMH has been used in hyperemesis gravidarum, either before or instead of ondansetron. There are studies on the use of DMH during gestation in rats (10). However, the effects of DMH on postnatal pups have not been addressed in these studies.

Pharmaceutical agents to be used during gestation should have a very high safety profile. Moreover, they should not have a negative effect on prenatal development of the fetus and postnatal development of the offspring. Some of the previous studies showed that pharmaceutical agents used as an antiemetic during gestation had negative effects (small birth weight, decreasing litter number, stillbirth, birth complications such as hypospadias, duodenal atresia and pulmonary stenosis) on morphometric development pre-and postnatally (1, 9, 11). However, these studies lack detailed morphometric data (e.g. measurements relative to skeletal system, especially) (11-13). A literature search revealed neither animal studies on the use

This study was presented as an abstract at the 11th international congress of anatomy, 2007 Denizli

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of ondansetron during gestation nor studies on the effects of prenatally administered DMH on postnatal morphometric development (e.g. eksternal measurement related to head, thorax, upper and lower limbs). The present study attempts to explore the effects of DMH and ondansetron that are commonly used as antiemetics during pregnancy on the length of gestation, maternal weight gain during gestation, and postnatal morphometric development of offspring.

Material and Methods

Thirty female (10-12 weeks old, weighing 139-165 g) and 15 male Wistar albino rats used for mating were obtained from the Experimental Animals Laboratory of Süleyman Demirel University and used in the study. Rats were maintained on a 12h: 12h light/dark (am.08.00, pm. 08.00) cycle, at room temperature and had free access to food and water. Approval from the Ethical Committee of Süleyman Demirel University School of Medicine has been established. All procedures were carried out in accordance with the guidelines on the care and use of laboratory animals set by the National Institute of Health, USA (NIH Publications No. 86-23, revised 1985).

Three groups of rats were formed. Group 1: control group (n: 10), group 2: DMH group (n: 10) and Group 3: ondansetron group (n: 10). To impregnate female rats, one male and two female rats were kept in the same cage for 24 hours. Pregnancy was confirmed by vaginal smear. Four rats in the control, three rats in the DMH, and six rats in the ondansetron group became pregnant. Pregnant rats were then housed in cages, with two rats in each cage, and the day was designated as day 1 of gestation. Rats in the DMH group (Group 2) were given intramuscular DMH at 115 mg/kg/day dose for a week starting on day 1 while rats in the ondansetron group (Group 3) were given intraperitoneal ondansetron at a dose of 10 mg/kg/day (9, 10, 14). Control group rats (Group 1) were treated with 0.9% NaCl during the same period. The cause of medication in the first week of pregnancy is the observation of nausea and vomiting in the first trimester of pregnancy, and medication in this period. During gestation, maternal weights of rats in all groups were recorded every other day from day 1 until the day of birth using DENSI DS-05 electronic scale. Each pregnant rat was placed in a separate cage on day 18. Length of gestation in all pregnant rats in the three groups and litter size were determined.

Sucking/rooting reflex, movement, color, anal and urethral openings and presence or absence of a malformation was assessed on newborn pups (15, 16). In addition, eye and ear opening times, tooth eruption time and the time of descent of the testes were also recorded (17, 18).

Morphometric growth parameters of pups were measured in the control, DMH and ondansetron groups. Measurement of morphometric growth parameters were carried out according to previously reported methods, using standard anthropometric reference points (19, 20). Measurements were made by the same observer, as the interobserver variability for the morphometric parameters was not significant in the preliminary study ($p>0.05$). Morphometric reference points were used for parameters that were measured for the first time in the present study (21, 22). Weights and other morphometric parameters of the pups were measured in newborn and lactation periods and adulthood at the same time of the day; once a week until week 5 and every fortnight until week 11.

Parameters measured in the present study were:

a. Pup weight: Measured using DENSI DS-05 electronic scale.
b. Head circumference (HC): The distance around the widest part of the skull passing from the glabella of the frontal bone, parietal tuber, and posterior-most point of the occipital bone.
c. Bi-parietal diameter (BPD): Transverse distance between the parietal tubers.
d. Skull length: Sagittal distance between glabella and the posterior-most point of the occipital bone.
e. Face length: Distance between glabella and the anterior-most point of the mandible.
f. Thorax circumference: Distance measured at the widest part of the thorax.
g. Thorax width: Transverse distance between two vertical planes passing through the outermost points of the thorax.
h. Crown-rump length (CRL): Distance between the vertex and the point where the tail started.
i. Naso-anal length: The distance between the tip of the nose and the midpoint of the anus.
j. Forearm length: Distance between the midpoint of the elbow joint and the tip of the longest digit on forelimb.
k. Leg length: Distance between the midpoint of the knee joint and the tip of the longest digit on hindlimb.
l. Bi-acetabular distance: Transverse distance between the greater trochanters.

Measurements in pups were carried out and assessed for three periods separately:

a. Parameters in the newborn period (day 1)
b. Parameters in the lactation period (days 7, 14 and 21)
c. Adulthood parameters (weeks 5, 7, 9 and 11). Further, parameters were measured in male and female pups separately after week 7.

Arithmetic means of all parameters of pups in the DMH, ondansetron and control groups and standard deviations associated with these means were calculated for each week. Also, means and standard deviations of these parameters with respect to sex were also determined at weeks 7, 9 and 11. Non-parametric tests were used for comparison of parameters in DMH, ondansetron and control groups due to insufficient number of cases in some groups. Kruskall-Wallis analysis of variance was used first to compare groups. As a result of this analysis, significant groups were compared pair wise by Mann-Whitney U test. Mann-Whitney U test (for separate comparisons within each group) were used for sex comparison of parametric data.

The Bonferroni correction was used to adjust the significance level. The relations between age (weeks) and all parameters were tested by Pearson's correlation test. Student-T test (totally for all cases) and Mann-Whitney U test (for separate

comparisons within each group) were used for sex comparison of parametric data.

Results

Mean maternal weight gain during gestation was determined (Figure 1). Accordingly, mean increases in maternal weights in pregnant rats in the DMH and ondansetron groups were less than the control group (total maternal weight gain: 50 g in the control group, 27 g in the DMH group and 49 g in the ondansetron group) (Figure 1).

When the length of gestation was analyzed in the three groups, we found that the length of gestation was 22 days in one rat in the DMH group and two rats in the ondansetron group, whereas it was 21 days for the remaining rats.

The litter numbers in the control, DMH and ondansetron groups were 35, 21 and 53 pups born to 4, 3 and 6 rats, respectively.

It was determined that none of the pups in the control, DMH or ondansetron groups had any pathology or abnormality with regard to sucking/rooting reflex, movement, color, anal and urethral openings, ear and eye opening times. Furthermore, birth complications such as spontaneous abortion were not noted in any of the three groups.

Morphometric growth parameters pertaining to total body, cranium, thorax and limbs of pups in the control, DMH and ondansetron groups were measured from day 1 until week 11, separately for the newborn and lactation periods and adulthood. Means and standard deviations of all parameters with respect to weeks obtained from all groups are presented in Tables 1 A-C. Tables 2 A, B, C and D show the arithmetic means and standard deviations of all parameters in all groups at weeks 7, 9 and 11 with respect to males and females. Comparisons of groups showed that many of the parameters measured in the DMH group were lower than the control group in all periods, whereas those measured in the ondansetron group were higher then the controls and these differences were statistically significant ($p<0.05$, Tables 1 A, B, C). Parameters measured at weeks 7, 9 and 11 were compared between rats of the same sex in the control, DMH and ondansetron groups (male-male, female-female comparisons) and there were significant differences between groups ($p<0.05$, Tables 2 A, B, C, D). There were also significant differences between males and females of the same group with respect to the parameters measured at weeks 7, 9 and 11. ($p<0.05$, Tables 2 A, B, C, D). There was also a very strong positive correlation between age and all parameters measured postnatally in all groups ($p<0.001$).

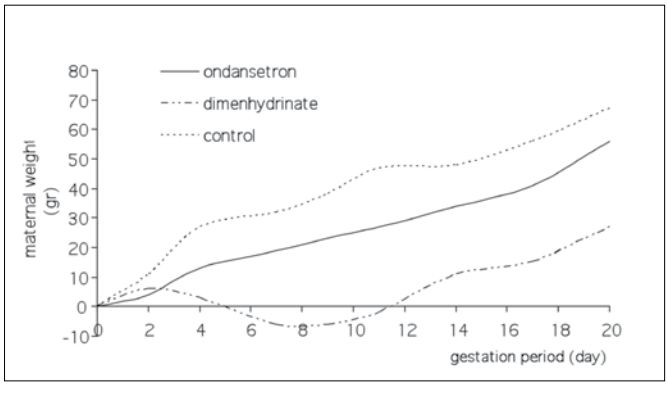

Figure 1. Changes in mean maternal weight gained by pregnant rats in the control, dimenhydrinate and ondansetron groups

Discussion

There are many factors affecting maternal weight gain during gestation. Maternal weight gain during pregnancy, on the other hand, has a direct impact on intra-uterine development and the effects of excessive or deficient maternal weight gain on prenatal and postnatal morphometric development have been established (23, 24). Previous studies on humans reported that steroids used as antiemetics during pregnancy can cause maternal weight loss, and this loss was statistically significant (25, 26). Another study on adult rats revealed that ondansetron caused less weight gain in males than females (9). To the best of our knowledge, there are no experimental studies on the gestational use of antiemetics on maternal weight gain in rats. In our study, we determined the mean maternal weight gained by pregnant rats during gestation. Weight gains during gestation in the DMH and ondansetron groups were less than the controls (Figure 1). Further, rats in the DMH group gained less weight than the ondansetron group. The fact that rats in the DMH and ondansetron groups gained little weight during gestation compared to the control group, may be due to the mechanisms related to stress induced during the administration of the drugs or the side effects of the drugs. Previous experimental studies also explained the low maternal weight gain with similar mechanisms (9, 14, 27-30). In the present study, maternal weight loss in the first week and increase in maternal weight gain following the cessation of the drug in the DMH group was noteworthy (Figure 1). Our finding that maternal weight gain with ondansetron was less than the controls is in agreement with the results of previous studies on adult rats (9). Moreover, the effect of DMH on maternal weight during gestation, in comparison to ondansetron, should be taken into consideration clinically.

Previous studies on humans reported that ondansetron and steroid given during pregnancy for antiemesis did not affect the length of pregnancy (1, 25, 31). There are no experimental studies that investigated the effects of antiemetics used during gestation on the length of gestation in rats. The length of gestation was 22 days in one rat in the DMH and two rats in the ondansetron groups while it was 21 days in the remaining rats. Statistical analyses showed that, compared to the control group, neither DMH nor ondansetron used as antiemetics altered the length of gestation significantly. In conclusion, our results are in agreement with the results of human studies.

When we looked at previous studies that investigated the effects of various medications administered during the fetal period, we found that non-steroidal anti-inflammatory drugs at toxic doses caused intrauterine growth retardation (32, 33) and nitric oxide synthase inhibitors resulted in fetal growth retardation by uteroplacental dysfunction (34, 35). Further, Ishida et al. (36) reported that DL-alpha-difluoromethyl orni-

Table 1a. Means (mm) and standard deviations of general morphometric parameters of pups in the Control (C), dimenhydrinate (DMH) and ondansetron (O) groups measured during newborn and lactation periods and adulthood

		General Parameters											
		N			Pup weight (gr)			Crown-rump length (CRL)			Naso-anal length		
		C	DMH	O	C	DMH	O	C	DMH	O	C	DMH	O
Newborn	first day	35	21	53	6.2±0.4	6.0±0.3	6.0±0.6	43.1±1.7[a]	38.8±1.0[b]	40.0±2.6[c]	48.8±2.2[a]	44.8±1.3[b]	46.1±2.9[c]
Lactation	1st week	27	14	43	14.1±2.6	13.2±0.7	13.3±2.4	54.7±2.9[a]	50.7±1.2[b]	56.6±3.0[c]	61.4±2.8[a]	59.1±1.5[b]	63.0±3.1[c]
	2nd week	27	14	43	19.0±2.7[a]	21.8±0.8	20.9±3.3[c]	63.7±2.2[a]	60.7±1.8[b]	66.1±4.4[c]	69.7±1.9[a]	72.9±1.3[b]	76.0±5.1[c]
	3rd week	27	14	43	28.2±3.8[a]	33.7±1.7[b]	33.5±5.4[c]	78.1±4.0[a]	73.0±1.5[b]	86.7±7.3[c]	90.0±3.3[a]	85.7±2.8[b]	98.2±7.7[c]
Adulthood	5th week	19	7	33	80.9±6.0[a]	88.0±4.0	87.7±7.9[c]	111.4±4.1[a]	88.2±2.3[b]	118.6±4.5[c]	121.8±5.0[a]	102.8±3.9[b]	131.9±5.5[c]
	7th week	19	7	33	124.7±6.4[a]	115.7±5.4[b]	134.7±11.3[c]	134.7±3.5[a]	101.0±3.0[b]	146.3±6.0[c]	146.3±4.3[a]	115.0±4.0[b]	160.0±7.1[c]
	9th week	19	7	33	152.8±16.4[a]	136.5±11.4[b]	166.2±17.3[c]	150.7±5.0[a]	114.7±2.6[b]	158.4±6.1[c]	163.4±6.2[a]	138.5±3.7[b]	171.3±6.6[c]
	11th week	19	7	33	198.4±32.7[a]	169.7±17.4[b]	217.2±34.2[c]	164.7±13.4[a]	130.7±3.4[b]	176.0±8.4[c]	180.5±16.9[a]	144.2±3.4[b]	190.1±10.1[c]

[a](p<0.05); Difference between Control and Dimenhydrinate groups
[b](p<0.05); Difference between Dimenhydrinate and Ondansetron groups
[c](p<0.05); Difference between Control and Ondansetron groups

Table 1b. Means (mm) and standard deviations of parameters pertaining to the cranium of pups in the Control (C), dimenhydrinate (DMH) and ondansetron (O) groups measured during newborn and lactation periods and adulthood

		Cranium Parameters											
		Head circumference			Bi-parietal diameter			Skull length			Face length		
		C	DMH	O	C	DMH	O	C	DMH	O	C	DMH	O
Newborn	first day	36.7±0.7	36.8±1.1[b]	37.6±1.6[c]	10.1±1.0	9.9±0.8	9.9±0.9	18.7±1.1[a]	15.8±0.7[b]	16.8±0.9[c]	10.0±0.9	8.9±0.6	10.6±10.9
Lactation	1st week	43.2±2.3	42.4±0.7[b]	44.5±2.1[c]	13.5±1.1	13.0±0.7	13.6±1.4	23.6±1.4[a]	22.0±0.9[b]	24.9±2.9[c]	12.9±0.9	12.1±0.6	12.8±1.5
	2nd week	50.7±2.2	49.7±1.4[b]	53.0±2.3[c]	17.0±0.9	16.9±0.7	16.7±1.2	30.8±2.0[a]	28.4±1.0[b]	33.2±2.5[c]	16.5±0.9	16.1±0.7	16.8±1.3
	3rd week	56.8±2.1	57.7±2.9[b]	59.3±2.3[c]	20.4±1.5	20.7±0.6	20.7±1.6	40.1±2.0[a]	35.7±0.7[b]	42.1±1.1[c]	19.8±1.3[a]	21.1±0.5	21.2±1.1[c]
Adulthood	5th week	66.4±1.3	65.8±0.8[b]	68.5±2.0[c]	27.6±1.2[a]	24.8±0.8[b]	27.0±1.0	50.5±1.4[a]	44.0±1.0[b]	49.7±1.2[c]	24.7±0.8[a]	26.2±1.3[b]	27.6±1.3[c]
	7th week	74.5±1.8[a]	71.4±0.9[b]	76.6±2.2[c]	33.5±5.0[a]	30.0±0.8[b]	33.6±1.1	55.5±1.5[a]	52.1±0.6[b]	54.5±1.3[c]	31.3±1.4[a]	30.1±1.0[b]	32.4±0.6[c]
	9th week	84.5±3.9[a]	75.5±0.9[b]	81.7±2.2[c]	37.7±1.8[a]	34.8±0.6[b]	36.3±1.6[c]	63.4±3.6[a]	61.1±1.4[b]	58.2±1.2[c]	34.0±1.2[a]	32.2±0.7[b]	35.5±1.4[c]
	11th week	92.3±2.6[a]	81.2±1.7[b]	85.3±1.8[c]	44.1±2.6[a]	38.1±1.0[b]	40.1±2.0[c]	74.2±1.9[a]	65.1±1.5[b]	61.6±1.7[c]	41.4±2.8[a]	37.0±1.0[b]	40.1±1.8[c]

[a](p<0.05); Difference between Control and Dimenhydrinate groups
[b](p<0.05); Difference between Dimenhydrinate and Ondansetron groups
[c](p<0.05); Difference between Control and Ondansetron groups

Table 1c. Means (mm) and standard deviations of parameters pertaining to the thorax and limbs of pups in the Control (C), dimenhydrinate (DMH) and ondansetron (O) groups measured during newborn and lactation periods and adulthood

		Thorax Parameters						Limbs Parameter								
		Thorax circumference			Thorax width			Forearm length			Leg lenght			Bi-acetabular distance		
		C	DMH	O	C	DMH	O	C	DMH	O	C	DMH	O	C	DMH	O
Newborn	first day	49.4±1.7[a]	46.5±1.8[b]	48.3±3.0[c]	12.4±0.8[a]	13.0±0.6[b]	14.6±1.0[c]	12.3±0.6[a]	11.4±0.5[b]	12.3±0.7	8.6±0.8	7.8±0.5	9.7±12.5	13.4±1.3	13.3±0.4	13.8±0.8
Lactation	1st week	55.0±2.3[a]	52.8±1.0[b]	56.7±3.5[c]	17.4±1.6[a]	16.2±0.9[b]	18.9±1.7[c]	15.7±1.3[a]	14.1±0.7[b]	15.9±2.6	11.2±1.5	10.2±0.6	11.5±2.6	18.1±1.2	17.5±0.8	17.7±1.5
	2nd week	63.3±2.3[a]	59.6±1.1[b]	66.4±3.4[c]	24.1±1.4[a]	20.0±0.6[b]	22.1±3.1[c]	20.9±1.3[a]	18.2±0.8[b]	22.9±1.0[c]	15.7±0.8[a]	12.8±0.7[b]	17.3±1.8[c]	25.4±1.7	23.9±1.2	26.0±4.1
	3rd week	73.2±3.2[a]	66.0±1.7[b]	81.5±5.0[c]	32.5±2.2[a]	25.5±1.0[b]	30.1±2.3[c]	27.1±1.8[a]	21.7±0.7[b]	28.5±2.0[c]	21.8±1.6[a]	16.5±0.8[b]	23.2±2.2[c]	33.6±3.0	33.1±2.1[b]	36.1±2.1[c]
Adulthood	5th week	88.1±2.4[a]	74.5±1.1[b]	100.3±3.9[c]	44.0±1.2[a]	31.8±1.8[b]	39.9±1.9[c]	33.7±0.7[a]	26.7±0.4[b]	38.0±1.3[c]	29.8±0.6[a]	20.2±0.7[b]	32.6±0.9[c]	49.0±1.3[a]	38.5±1.9[b]	50.0±1.8[c]
	7th week	101.1±4.7[a]	82.5±1.3[b]	123.6±6.4[c]	50.3±1.5[a]	36.7±1.4[b]	55.3±0.9[c]	38.3±0.8[a]	31.2±0.9[b]	41.4±0.5[c]	33.0±0.5[a]	24.1±0.6[b]	36.8±0.6[c]	54.6±3.3[a]	42.5±2.5[b]	60.3±2.2[c]
	9th week	116.7±7.9[a]	92.5±3.0[b]	140.6±7.6[c]	59.9±2.9[a]	42.8±1.7[b]	58.7±1.2[c]	42.4±1.0[a]	35.8±0.6[b]	42.9±0.7[c]	36.8±0.9[a]	28.2±0.7[b]	38.1±0.9[c]	61.8±4.0[a]	50.8±1.4[b]	64.6±1.9[c]
	11th week	139.2±12.6[a]	101.8±3.0[b]	157.4±7.7[c]	62.1±4.6[a]	51.2±1.3[b]	65.5±3.6[c]	44.5±1.0[a]	41.5±0.5[b]	45.4±1.7[c]	39.2±0.7[a]	32.2±0.4[b]	40.7±2.0[c]	65.0±4.0[a]	59.7±2.3[b]	71.7±4.4[c]

[a](p<0.05); Difference between Control and Dimenhydrinate groups
[b](p<0.05); Difference between Dimenhydrinate and Ondansetron groups
[c](p<0.05); Difference between Control and Ondansetron groups

Table 2a. Means (mm) and standard deviations of general morphometric parameters of male (m) and female (f) pups in the Control (C), dimenhydrinate (DMH) and ondansetron (O) groups measured during adulthood

	General Parameters																	
	Pup weight* (gr)						Crown-rump length* (CRL)						Naso-anal length*					
	C		DMH		O		C		DMH		O		C		DMH		O	
Sex (n)	m (9)	f (10)	m (2)	f (5)	m (20)	f (13)	m (9)	f (10)	m (2)	f (5)	m (20)	f (13)	m (9)	f (10)	m (2)	f (5)	m (20)	f (13)
7th week	129.2±6.6[a]	120.8±2.6	111.0±4.2[b]	117.6±4.8	142.6±5.5[c]	122.6±5.7	136.1±4.1[a]	133.5±2.4[a]	98.5±2.1[b]	102.0±3.0[b]	150.0±4.5[c]	140.8±3.2[c]	147.2±5.6[a]	145.5±2.8[a]	112.5±3.5[b]	116.0±4.1[b]	164.0±5.5	153.8±4.6
9th week	167.8±10.5[a]	139.3±3.4	124.0±15.5[b]	141.6±5.5	178.0±8.9[c]	148.0±9.0[c]	155.0±2.5[a]	147.0±3.4[a]	111.5±2.1[b]	116.0±1.4[b]	162.5±3.4[c]	152.1±3.4[c]	167.7±5.0[a]	159.5±4.3[a]	137.5±3.5[b]	139.0±4.1[b]	175.2±4.9[c]	165.3±3.7
11th week	231.1±7.8[a]	169.0±8.0	149.5±14.8[b]	177.8±107	242.8±9.3[c]	177.9±14.8	178.3±2.5[a]	152.5±2.6[a]	127.5±3.5[b]	132.0±2.7[b]	182.0±3.7[c]	167.0±4.7[c]	197.2±5.6[a]	165.5±4.3[a]	142.5±3.5[b]	145.0±3.5	197.3±4.6[c]	179.2±5.3

* Within-group comparisons of males and females: Significant in control and ondansetron groups but not significant in dimenhydrinate group at weeks 7, 9 and 11.
Comparison of rats of same sex between groups (male-male, female-female)
[a](p<0.05); Difference between Control and Dimenhydrinate groups
[b](p<0.05); Difference between Dimenhydrinate and Ondansetron groups
[c](p<0.05); Difference between Control and Ondansetron groups

Table 2b. Means (mm) and standard deviations of parameters pertaining to the cranium of male (m) and female (f) pups in the Control (C), dimenhydrinate (DMH) and ondansetron (O) groups measured during adulthood

	Cranium Parameter																					
	Head circumference*						Bi-parietal diameter*						Skull length*						Face lenght*			
	C		DMH		O		C		DMH		O		C		DMH		O		C		DMH		O	
Sex (n)	m (9)	f (10)	m (2)	f (5)	m (20)	f (13)	m (9)	f (10)	m (2)	f (5)	m (20)	f (13)	m (9)	f (10)	m (2)	f (5)	m (20)	f (13)	m (9)	f (10)	m (2)	f (5)	m (20)	f (13)
7th week	75.6 ± 1.9[a]	73.6 ± 0.6[a]	70.5 ± 0.7[b]	71.8 ± 0.8[b]	78.0 ± 1.7	74.5 ± 0.6	34.7 ± 0.8[a]	32.5 ± 0.5[a]	30.0 ± 1.4[b]	30.0 ± 0.7[b]	34.3 ± 0.9[c]	32.6 ± 0.5	56.7 ± 1.0[a]	54.5 ± 0.9[a]	51.5 ± 0.7[b]	52.4 ± 0.5[b]	54.9 ± 1.6[c]	54.0 ± 0.7[c]	32.2 ± 1.0[a]	30.6 ± 1.2[a]	30.5 ± 0.7[b]	30.0 ± 1.2[b]	32.6 ± 0.5[c]	32.1 ± 0.6[c]
9th week	88.1 ± 1.2[a]	81.3 ± 2.4[a]	75.0 ± 1.4[b]	75.8 ± 0.8	83.3 ± 0.6[c]	79.3 ± 1.5[c]	39.3 ± 0.7a	36.3 ± 1.2	34.5 ± 0.7	35.0 ± 0.7[b]	37.2 ± 1.6[c]	35.1 ± 0.8[c]	66.8 ± 1.0[a]	60.4 ± 1.7[a]	59.5 ± 0.7[b]	61.8 ± 1.0[b]	59.0 ± 0.7[c]	57.1 ± 1.0[c]	34.8 ± 1.0[a]	33.2 ± 0.9[a]	32.0 ± 1.4[b]	32.4 ± 0.5[b]	36.4 ± 0.9[c]	34.2 ± 1.0[c]
11th week	94.6 ± 1.0[a]	90.2 ± 1.4[a]	80.5 ± 2.1[b]	81.6 ± 1.8	86.6 ± 0.9[c]	83.4 ± 0.9[c]	46.5 ± 1.1[a]	42.0 ± 1.5[a]	37.0 ± 0.0	38.6 ± 0.8[b]	41.5 ± 0.8[c]	38.0 ± 1.3[c]	76.0 ± 1.0[a]	72.6 ± 0.9[a]	63.5 ± 2.1[b]	65.8 ± 0.8[b]	62.7 ± 1.1[c]	59.8 ± 1.0[c]	44.0 ± 0.8[a]	39.1 ± 1.5[a]	36.0 ± 1.4[b]	37.4 ± 0.5[b]	41.3 ± 1.2[c]	38.2 ± 0.8[c]

*Within-group comparisons of males and females: Significant in control and ondansetron groups but not significant in dimenhydrinate group at weeks 7, 9 and 11
Comparison of rats of same sex between groups (male-male, female-female)
[a](p<0.05); Difference between Control and Dimenhydrinate groups
[b](p<0.05); Difference between Dimenhydrinate and Ondansetron groups
[c](p<0.05); Difference between Control and Ondansetron groups

thinen (DFMO) altered the mother's nutrition during gestation, caused placental dysfunction and fetal growth retardation. In another study, researchers observed decreases in placental mass and fetal weight and fetal growth retardation following dexamethasone treatment (37). Einarson et al. (1) argued that ondansetron, when used as an antiemetic during pregnancy, can cause stillbirth and birth complications such as hypospadias, duodenal atresia and pulmonary stenosis in newborn humans. Moreover, though statistically insignificant in comparison to the control groups, birth complications including spontaneous abortion with antiemetic treatment have been reported (1, 9, 14, 38, 39). On the other hand, studies also exist reporting that DMH and ondansetron used for treating hyperemesis gravidarum in humans had no effect on fetal development or adverse effects on the newborn (9, 11, 29, 38, 39). Pups born to rats in the DMH, ondansetron and control groups had normal sucking/rooting reflex, movement, color, anal and urethral openings and eye and ear opening times and none of them had any birth complications. Results obtained in the present study are in agreement with the results of human studies (9, 11, 29, 38, 39).

There are no studies in the literature that addressed the postnatal growth parameters pertaining to the body, cranium, thorax and limbs when antiemetics are administered during gestation (21). It has been reported that lithium use in pregnant women could cause a macrosomic fetus, and lithium's effects could also be observed in the postnatal period (40, 41). We measured the postnatal growth parameters on the body, cranium, thorax and limbs in pups of DMH, ondansetron and control groups between day 1 and week 11. Comparison of parameters in the ondansetron and control groups showed that, in all periods, the majority of the parameters were significantly higher in the ondansetron group, and especially in male offspring, compared to the control group ($p<0.05$, Tables 1 A, B, C). Morphometric parameters of pups in the DMH and control groups in the postnatal period were compared and pups in the DMH group were found to be less developed than the control group ($p<0.05$, Tables 1 A, B, C). When the development of pups in the DMH and ondansetron groups was compared, we found significant differences between the two groups, with greater parameters in the ondansetron group ($p<0.05$, Tables 1 A, B, C). In short, parameters in the ondansetron group were greater; parameters in the DMH group were less than the controls. These findings suggest that ondansetron increases the rate of postnatal morphometric development, while DMH exerted a negative effect. We believe that postnatal growth retardation is caused by the toxic and metabolic effects of prenatal DMH injection and its effect on uteroplacental circulation which, consequently, has an impact on fetal development. It must be taken into consideration that mean values for the birthweight and morphometric parameters of postnatal development may be influenced by the difference in the number of pups per mother rat. We were unable to comment on why the parameters in the ondansetron group were greater than the controls. This emphasizes that further studies are required to investigate the effects of prenatal administration of DMH and ondansetron on pre- and postnatal development.

Table 2c. Means (mm) and standard deviations of parameters pertaining to the thorax of male (m) and female (f) pups in the Control (C), dimenhydrinate (DMH) and ondansetron (O) groups measured during adulthood

Thorax Parameters												
	Thorax circumference*						Thorax width*					
	C		DMH		O		C		DMH		O	
Sex (n)	m (9)	f (10)	m (2)	f (5)	m (20)	f (13)	m (9)	f (10)	m (2)	f (5)	m (20)	f (13)
7th week	104.5±4.4[a]	98.0±2.3[a]	81.0±1.4[b]	83.2±0.8[b]	127.9±4.3[c]	117.2±2.6[c]	51.4±1.2[a]	49.3±0.9[a]	36.0±1.4[b]	37.0±1.5[b]	55.6±0.9c	55.0±0.7[c]
9th week	123.8±4.8[a]	110.3±2.9[a]	88.5±2.1[b]	94.2±1.0[b]	146.1±3.4[c]	132.1±2.7[c]	62.7±1.3[a]	57.4±0.8[a]	40.5±0.7[b]	43.8±0.8[b]	59.1±1.4	58.1±0.6[c]
11th week	151.1±4.8[a]	128.5±5.2[a]	99.0±1.4[b]	103.0±2.7[b]	163.0±2.9	149.0±4.1[c]	66.5±1.7[a]	58.2±1.8[a]	50.0±0.0[b]	51.8±1.3[b]	68.1±1.1[c]	61.1±1.5

*Within-group comparisons of males and females: Significant in control and ondansetron groups but not significant in dimenhydrinate group at weeks 7, 9 and 11
Comparison of rats of same sex between groups (male-male, female-female)
[a](p<0.05); Difference between Control and Dimenhydrinate groups
[b](p<0.05); Difference between Dimenhydrinate and Ondansetron groups
[c](p<0.05); Difference between Control and Ondansetron groups

Table 2d. Means (mm) and standard deviations of parameters pertaining to the limbs of male (m) and female (f) pups in the Control (C), dimenhydrinate (DMH) and ondansetron (O) groups measured during adulthood

Limbs Parameter																		
	Forearm length*						Leg length*						Bi-acetabular distance*					
	C		DMH		O		C		DMH		O		C		DMH		O	
Sex (n)	m (9)	f (10)	m (2)	f (5)	m (20)	f (13)	m (9)	f (10)	m (2)	f (5)	m (20)	f (13)	m (9)	f (10)	m (2)	f (5)	m (20)	f (13)
7th week	38.8±0.6[a]	37.8±0.6[a]	31.5±0.7[b]	31.2±1.0[b]	41.5±0.5[c]	41.3±0.5[c]	33.2±0.6[a]	32.8±0.4[a]	24.5±0.7[b]	24.0±0.7[b]	37.1±0.6[c]	36.3±0.5[c]	57.1±2.1[a]	52.5±2.6	42.5±3.5[b]	42.6±2.5[b]	60.6±2.5[c]	59.9±2.0[c]
9th week	43.3±0.5[a]	41.7±0.6[a]	36.5±0.7[b]	35.6±0.5[b]	43.4±0.5[c]	42.3±0.4[c]	37.7±0.4[a]	36.1±0.5[a]	28.0±1.4[b]	28.4±0.5[b]	38.6±0.8	37.4±0.5[c]	65.5±1.0[a]	58.5±2.4[a]	50.0±0.0[b]	51.2±1.6	64.4±2.1[c]	64.9±1.7[c]
11th week	45.4±0.5[a]	43.7±0.4[a]	42.0±0.0[b]	41.4±0.5[b]	46.8±0.6[c]	43.3±0.5	39.7±0.4[a]	38.7±0.4	32.0±0.0[b]	32.4±0.5[b]	42.3±0.4[c]	38.3±0.5[c]	68.3±2.5	62.0±2.5[a]	57.5±3.5	60.6±1.3	75.0±1.5[c]	66.7±1.8[c]

* Within-group comparisons of males and females: Significant in control and ondansetron groups but not significant in dimenhydrinate group at weeks 7, 9 and 11
Comparison of rats of same sex between groups (male-male, female-female)
[a](p<0.05); Difference between Control and Dimenhydrinate groups
[b](p<0.05); Difference between Dimenhydrinate and Ondansetron groups
[c](p<0.05); Difference between Control and Ondansetron groups

There are very few studies addressing the sex differences in ondansetron's effects. Only Tucker et al. (9) showed that ondansetron administration to adult rats resulted in less weight gain in male rats compared to females. When we compared the postnatal parameters in males with those in females in each group, we found that male rats in the control and ondansetron groups had significantly greater parameters ($p<0.05$, Tables 2 A, B, C, D). There was no significant difference in the DMH group ($p<0.05$, Tables 2 A, B, C, D). When parameters obtained in rats of the same sex were compared between groups, we determined sex differences in most of the parameters except weight. Comparison of postnatal weight of female rats did not show a group difference, while males did (Tables 2 A, B, C, D). This result led us to conclude that DMH and ondansetron may have different effects in males and females with regard to weight. This result contradicts the results of Tucker at al. (9) who reported that ondansetron caused less weight gain in male rats, since our study found that the effect of ondansetron was different for males and females with regard to weight. Therefore, new studies are needed to examine whether ondansetron has different effects on males and females regarding other parameters. We conider that, as well as the low number of pregnant rats in some groups, evaluating our study as a pioneer in this subject would be valuable. At the same time, studies on about Ondansetron and DMH in prenatal and postnatal periods are necessary.

In conclusion, DMH used as an antiemetic during gestation has adverse effects on the postnatal development of offspring. Nevertheless, new research is necessary to determine the postnatal effects of drugs taken during gestation for treatment of any ailment.

Conflict of Interest

No conflict of interest was declared by the authors.

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