Yonago Acta Medica 2020;63(4):301–307 doi: 10.33160/yam.2020.11.012 *Original Article*

Plasma Oxytocin Concentrations During and After Gestation in Japanese Pregnant Women Affected by Anxiety Disorder and Endometriosis

Toshio Masumoto,* Kazunari Onishi,† Tasuku Harada,‡ Hiroki Amano,* Shinji Otani§ and Youichi Kurozawa*

**Division of Health Administration and Promotion, Department of Social Medicine, Faculty of Medicine, Tottori University, Yonago 683-8503, Japan, †Division of Environmental Health Sciences, Graduate School of Public Health, St. Luke's International University, Tokyo 104-0045, Japan, ‡Department of Obstetrics and Gynecology, Research Center for Bioscience and Technology, Faculty of Medicine, Tottori University, Yonago 683-8503, Japan, and §International Platform for Dryland Research and Education, Tottori University, Tottori 680-0001, Japan*

ABSTRACT

Background Oxytocin has a key role in mother-infant bonding, maternal care, social interaction, and stress-related psychiatric disorders. However, the factors determining oxytocin concentrations during and after pregnancy such as medical history related to nursing or parental behavior are unknown. To elucidate these, we analyzed the relationships between oxytocin concentrations during and after pregnancy, and medical history assessed in the Japan Environment and Children's Study (JECS).

Methods We then selected the pregnant women with a medical history of anxiety disorder and endometriosis as cases and pregnant women without medical history as controls adjusting the cohort for age and parity for a nested case-control study, after which 162 women remained for analysis. We evaluated 162 pregnant women from JECS using answers provided in a questionnaire and by measuring plasma oxytocin concentration by ELISA during the first (T1) and second (T2) trimesters of pregnancy, and after childbirth (T3).

Results Oxytocin concentration increased in a time dependent manner, consistent with previous reports. There were weak negative correlations between oxytocin concentration at T1 and the mother's age and height, but no correlation with other factors. The mean oxytocin concentrations of pregnant women with a history of an anxiety disorder ($n = 7$) and endometriosis ($n = 13$) were significantly lower than those of pregnant women with no such history at T2 and T3.

Conclusion These results suggest that oxytocin concentrations during and after pregnancy were affected by a past history of anxiety disorder and endometriosis. This is the first study of the relationship between oxytocin concentration and endometriosis. To elucidate the molecular mechanisms, further study is needed.

Key words anxiety disorder; endometriosis; oxytocin

Post-partum depression (PPD) is defined by the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition, as a major depressive episode occurring within 4 weeks to 3–6 months postpartum.[1] Ten to 15% of mothers develop this disorder during the first 6 months after childbirth.[2] PPD affects child development by impairing mother-infant bonding and increasing the risk of developmental disorders in infants.[3–5] Because a poor mother-infant relationship increases the risk of psychological illness in the infants,[6, 7] it is important to identify risk factors for PPD to prevent such deficits in the mother-infant relationship.

Oxytocin is a candidate mediator of the association between poor social bonding and subsequent stress-related psychiatric disorders. It is a peptide hormone that is essential for mammalian labor and lactation.[8] Recently, a number of studies have shown that oxytocin has functions not only in peripheral tissues, but also in the central nervous system, affecting brain functions, such as memory, social bonding, and maternal behavior.[9–16] For example, oxytocin was shown to be associated with autism in human studies, and patients who were given oxytocin were more likely to trust other people.[17–19] Interestingly, oxytocin receptor knock-out mice showed impaired mother-infant bonding, manifesting in infanticide and impaired parental behavior.[20] These findings indicate that oxytocin has an important role in mother-infant relationships, child mental development and parental behavior, in addition to its known physiological roles, such as in promoting milk ejection. Although oxytocin now appears to have a role in constructing the mother-infant relationship, it is unknown whether the maternal circulating concentration of oxytocin affects social bonding. In particular,

Corresponding author: Toshio Masumoto, PhD
tmasumoto@tottori-u.ac.jp
Received 2020 September 10
Accepted 2020 October 2
Online published 2020 November 5
Abbreviations: ELISA, Enzyme-Linked ImmunoSorbent Assay; JECS, Japan Environment and Children's Study; MOE, Ministry of Environment; PPD, post-partum depression

the factors affecting maternal oxytocin levels during and after pregnancy such as medical history related to nursing behavior are poorly characterized. To address this deficiency in knowledge, we analyzed the relationships between oxytocin concentration during and after pregnancy and questionnaire in the Japan Environment and Children's Study (JECS).

MATERIALS AND METHODS

Data sources

The purpose of the JECS, an ongoing prospective birth cohort study that began in 2011, is to evaluate the impact of various environmental factors on children's health and development.[21, 22] The JECS protocol was approved by the Institutional Review Board on epidemiological studies of the Ministry of the Environment (MOE) and the ethics committees of all participating institutions. The JECS was conducted in accordance with the Declaration of Helsinki, 1964, and its later amendments, and with other national regulations. The present study was based on the jecs-ag-ai-20160424 dataset released in April 2016, which does not contain any patient identifying information.

Self-administered questionnaires, which were completed by the women during their first and second/third trimesters of pregnancy and after child birth, were used to collect information on the age, body mass, and height of the mother, the body mass of the child, maternal parity, and her medical history of psychiatric disorder and gynopathy at the first trimester.

The pregnant participants were regularly examined in the antenatal clinic, and blood samples were drawn for routine gynecological checks during the first trimester (15.7 ± 3.6 weeks of gestation, T1), the second trimester (25.6 ± 2.0 weeks of gestation, T2), and after childbirth (3.4 ± 0.9 days postpartum, T3). All blood samples were collected into tubes containing EDTA. Because the blood samples were to be used in the main JECS study, residual blood in the EDTA-containing tubes was used for this study if available. Residual blood was fractionated to generate plasma and erythrocytes by centrifugation. Plasma samples were sent to Special Reference Laboratories, Inc. (Tokyo, Japan) for measurement of oxytocin concentration by Enzyme-Linked ImmunoSorbent Assay (ELISA).

Study subjects

Pregnant women who visited antenatal hospitals in Tottori Prefecture were initially recruited to JECS, and JECS participants in the coastal area of the prefecture (3,099 pregnant women) were considered for recruitment into the adjunct study, of which 840 were recruited. We then selected the pregnant women with medical history of an anxiety disorder and endometriosis as case and pregnant women without medical history as control for adjusting the cohort for age and parity for nested case-control study, after which 162 women remained for analysis. The women were then classified by their medical history of psychiatric disorders or gynopathy.

Statistical analysis

Pearson's correlation coefficient was used to evaluate the relationship between plasma oxytocin concentration and maternal parameters, and Student's *t*-test for comparison of oxytocin concentrations between groups, using IBMSPSS software version 23 (IBM, Armonk, NY) and MATLAB (Mathworks, Natick, MA). $P < 0.05$ was considered to represent statistical significance. MATLAB software was used to prepare the graphs.

Ethical approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments, or comparable ethical standards. This article does not contain any studies with animals performed by any of the authors. The study protocol was approved by Tottori University (No. 2203) and this study was conducted under the approval for JECS as an adjunct study. Written consent was obtained from MOE on the basis that it did not interfere with the main JECS study.

RESULTS

We collected 162 blood samples from JECS participants in the Tottori Regional Center. Their mean age was 31, mean height was 156 cm, mean body mass before pregnancy was 52 kg, mean body mass before childbirth was 62 kg, and their mean number of pregnancies was 2 (Table 1). First, plasma oxytocin concentration was quantified by ELISA. The mean oxytocin concentration was 85 micro unit/mL at T1, 396 micro unit/mL at (T2), and 670 micro unit/mL after childbirth (T3); i.e. oxytocin concentration increased in a time-dependent manner (Fig. 1). However, oxytocin concentrations did not increase in some patients. To elucidate the factors affecting oxytocin concentration during and after pregnancy, correlation coefficients were calculated. The variables used were oxytocin concentration, body mass, height, age, and parity. There was a weak negative correlation between oxytocin concentration at T1 and the mother's age and height, but there was no correlation with other factors (Table 1).

Next, to determine why oxytocin concentrations might differ between women at time-points during and

Table 1. Sample characteristics for the whole population and correlations between parameters and oxytocin concentrations

	Average (SD)	ρ (T1)	*P*	ρ (T2)	*P*	ρ (T3)	*P*
Age	31.3 (5.4)	–0.240*	0.017	0.011	0.9	0.122	0.177
Height (cm)	156 (5.47)	–0.188*	0.033	0.095	0.232	–0.05	0.534
Body mass before pregnancy (kg)	51.6 (7.59)	–0.04	0.664	–0.117	0.15	–0.012	0.883
Body mass before delivery (kg)	61.5 (8.45)	–0.025	0.777	–0.177	0.026	0.066	0.41
Parity	2.0 (1.2)	0.003	0.973	0.072	0.367	0.114	0.154

Mean values for the whole population and correlation coefficients between plasma oxytocin concentration and each parameter were calculated. * indicates a significant correlation. Total $n = 162$. *$P < 0.05$

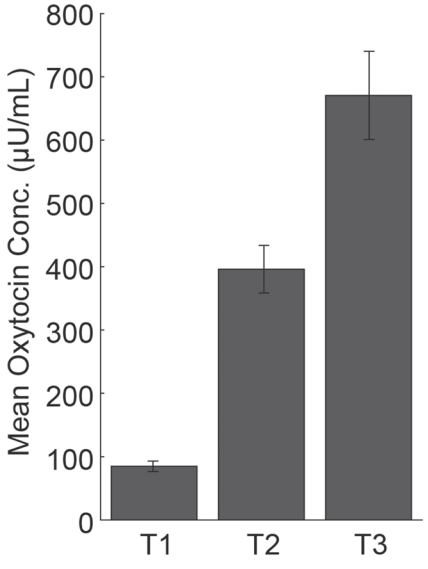

Fig. 1. Time course of plasma oxytocin concentration in mothers. Plasma oxytocin increased during pregnancy in a time-dependent manner. Blood samples were collected during the first (15.7 ± 3.6 weeks of gestation, T1) and second (25.6 ± 2.0 weeks of gestation, T2) trimester of pregnancy, and after childbirth (3.4 ± 0.90 days postpartum, T3). Plasma samples were analyzed by ELISA. Values are mean ± SEM.

after pregnancy, patients were classified according to their medical history, and mean oxytocin concentrations were calculated and compared between groups. Specifically, psychiatric disorders and gynopathy were considered (Table 2). The oxytocin concentrations of pregnant women with a history of an anxiety disorder were significantly lower than those of women without this history during the second trimester and after childbirth (Fig. 2A, Table 2). However, there were no correlations between oxytocin and other psychiatric disorders (Table 2). With respect to gynopathy, the oxytocin concentrations of pregnant women with a history of endometriosis were also lower than those of women without such history (Fig. 2B, Table 2). An association with oxytocin was not detected for any other gynopathies (Table 2). These results indicate that a medical history of an anxiety disorder and endometriosis is associated with oxytocin concentration during and after pregnancy.

DISCUSSION

In previous studies, it has been shown that circulating oxytocin concentrations are associated with the likelihood of a mother becoming depressed during and after pregnancy, and the social bonding between mother and children.[20, 21] However, the factors affecting oxytocin concentration in mothers, such as medical history are poorly characterized. Therefore, to identify some of these factors, we analyzed the relationships between oxytocin concentration during and after pregnancy with various parameters in subjects enrolled in JECS. We found that oxytocin concentration increased in a time-dependent manner (Fig. 1) and that a medical history of an anxiety disorder and endometriosis was associated with lower oxytocin concentrations during the second trimester of pregnancy and after childbirth (Fig. 2).

In clinical studies, it has been shown that plasma oxytocin concentrations in women gradually increase from 25th weeks of pregnancy.[23, 24] Consistent with these, oxytocin concentrations increased in a time-dependent manner in our study (Fig. 1).

Recent studies have suggested that oxytocin may have anxiolytic effects. Oxytocin released from the hypothalamus mediates mating-induced anxiolysis in rats, and in human studies intranasal oxytocin has been shown to reduce anxiety, possibly through effects at the level of the amygdala.[17, 25–29] In our study, women who had a medical history of an anxiety disorder had lower

Table 2. Mean oxytocin concentrations in women classified according to their medical history

Medical history		*N*	Average oxytocin concentration (μU/mL)					
			T1 (SEM)	*P*	T2 (SEM)	*P*	T3 (SEM)	*P*
Psychiatric disorder								
Depression	w/	6	65.1 ± 9.3	0.197	311 ± 117	0.500	526 ± 236	0.565
	w/o	158	85.4 ± 7.6		399 ± 39.0		676 ± 71.1	
Dysautonomia	w/	12	106 ± 44.9	0.722	338 ± 96.7	0.554	523 ± 151	0.402
	w/o	50	83.5 ±7.31		401 ± 40.1		681 ± 73.2	
Anxiety disorder	w/	7	147 ± 54.3	0.318	183 ± 43.4*	0.001	252 ± 71.4*	< .001
	w/o	155	81.7 ± 7.24		406 ± 39.2		690 ± 71.6	
Epilepsy	w/	3	46.9 ± 22.7	0.221	169 ± 90.8	0.108	163 ± 103*	0.012
	w/o	159	85.7 ± 7.48		400 ± 38.3		680 ± 70.0	
Migraine	w/	11	108 ± 37.5	0.547	450 ± 148	0.715	859 ± 331	0.563
	w/o	151	82.8 ± 7.4		392 ± 39		657 ± 70.1	
Others	w/	6	104 ± 57.1	0.736	426 ± 227	0.897	604 ± 439	0.881
	w/o	156	83.8 ± 7.32		395 ± 38.4		673 ± 69.9	
Gynopathy								
Menstrual irregularity	w/	22	105 ± 33.7	0.576	631 ± 158	0.107	944 ± 228	0.196
	w/o	140	81.9 ± 6.85		359 ± 35.3		626 ± 70.9	
Endometriosis	w/	13	40.1 ± 12.5*	0.005	194 ± 44.2*	< .001	148 ± 15.3*	< .001
	w/o	149	89.4 ± 7.9		414 ± 40.6		702 ± 73.2	
Uterine fibroid	w/	8	55.5 ± 17.9	0.176	231 ± 108	0.166	755 ± 475	0.869
	w/o	154	86.5 ± 7.68		405 ± 39.2		667 ± 69.0	
Ovarian tumor	w/	5	84.0 ± 27.2	0.982	625 ± 315	0.497	994 ± 488	0.534
	w/o	157	84.8 ± 7.55		389 ± 37.7		660 ± 69.6	
Others	w/	11	53.1 ± 11.8*	0.045	431 ± 176	0.838	545 ± 207	0.550
	w/o	151	87.2 ± 7.81		394 ± 38.6		680 ± 72.5	

* indicates a significant difference from mothers with no relevant medical history. Because the number of patients with a particular medical history were < 2, the mean oxytocin concentration was not calculated in medical history of Schizophrenia, Meningitis, Hydrocephalus, Uterine adenomyosis, Malformation of Uterus, Polycystic Ovary Syndrome and Malformation of Urinary Tract or Genital Organs. 'w' and 'w/o' indicate the with and without, respectively. SEM, standard error of the mean.

oxytocin concentrations than those who did not (Fig. 2A). Taking all these data together, oxytocin concentrations may be lower in individuals with a history of anxiety during pregnancy, because their oxytocin secretory capacity is lower than in women without this history (Fig. 3).

This is the first study of the relationship between oxytocin concentration and endometriosis. It is reported that estrogen activity was enhanced in endometriosis tissue.[30] In addition, sex hormone such as estradiol is increased oxytocin receptor expression in vitro study.[31] In previous studies, endometriosis patients tend to abnormally express oxytocin receptor higher in the uterine junctional zone compared with normal patients.[32, 33] These results indicate that oxytocin receptor expression is increased by enhancement of sex hormone activity due to endometriosis. Due to the increase of oxytocin receptor by endometriosis, oxytocin sensitivity in the uterus may be enhanced and lower oxytocin is sufficient for the physiological role of oxytocin. This mechanism may provide an explanation for the lower concentrations of oxytocin measured during and after pregnancy (Fig. 3B). However, because the molecular system of oxytocin regulation in endometriosis patients is still unknown, further study is needed.

Previous clinical studies have shown that oxytocin concentrations are associated with PPD, and in a genetics study, a polymorphism in the oxytocin gene at rs2740210 was associated with PPD.[3, 34–36] In recent intervention studies, intranasal oxytocin treatment of

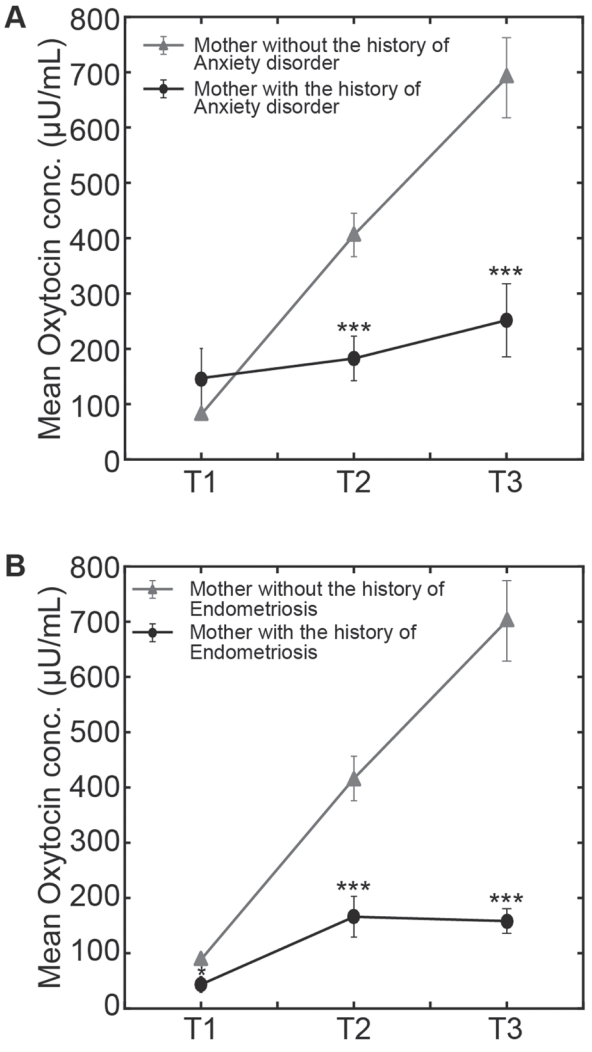

Fig. 2. Time course of plasma oxytocin concentration in mothers with a medical history. Patients were classified by their medical history and their mean plasma oxytocin concentrations were calculated. Anxiety disorder (**A**) and endometriosis (**B**). Values are mean ± SEM. ***$P < 0.001$; *$P < 0.05$, by *t*-test.

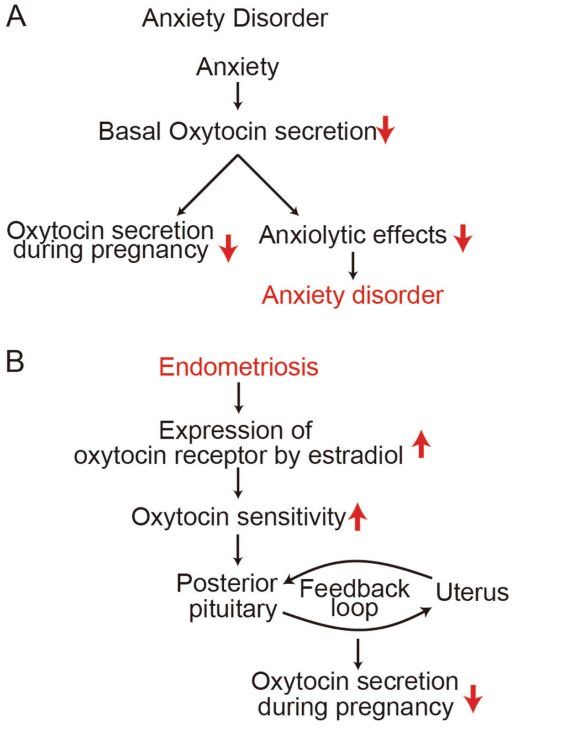

Fig. 3. Possible mechanism underlying the relationship between oxytocin concentration during pregnancy and medical history. (**A**) Basal oxytocin level was lower in mothers who had a history of an anxiety disorder. Due to inhibition of basal oxytocin secretion, an anxiety disorder developed. This inhibition affects the plasma oxytocin concentration during pregnancy. (**B**) Oxytocin receptor expression is affected by a maternal history of endometriosis. This change in expression affects the feedback from the uterus to the brain, resulting in lower plasma oxytocin during pregnancy.

PPD patients was shown to partially ameliorate affective disturbances and to improve the mother-infant relationship.[35, 37] These results are consistent with an effect of oxytocin on PPD. In addition, a medical history of endometriosis has been shown to be a risk factor for PPD.[38] In our study, a medical history of endometriosis was associated with lower oxytocin concentrations during and after pregnancy (Figs. 2B and 3B). Although molecular mechanisms linking a medical history of endometriosis with PPD are unknown, oxytocin is likely to play a role in the PPD developing mechanism by medical history of endometriosis.

There were four principal limitations to our study. The first was that we could not judge the severity of endometriosis perfectly because questionnaires were self-administered. It is possible that the severity of endometriosis may affect oxytocin secretion. To elucidate this, further studies are needed. The second was that we could not match the timing of blood sampling between participants. Blood samples were collected in the daytime, but circadian rhythms may still affect the measured oxytocin concentrations. However, in previous clinical studies, no time-dependent peaks in plasma oxytocin were identified in humans during the day.[39, 40] These data indicate that there is no influence from circadian rhythms with respect to plasma oxytocin concentration. Thus, we did not anticipate any effect of sample timing on our results. The third limitation was the storage condition of the blood samples. We stored the blood samples with EDTA at −20°C, without the inclusion of a protease inhibitor; therefore, oxytocin levels may have decreased during storage. However, oxytocin is highly stable in human plasma for 1 month and at least 17 hours when frozen or in cold aqueous solution, respectively,[41–44] implying that oxytocin concentration should

not be affected in samples frozen without a protease inhibitor for the relevant period of time. The fourth limitation was that we could not identify an effect of changes in an individual mother's oxytocin concentration on her mental health and behavior. However, in an animal study, oxytocin knock-out mice showed altered parental behavior,[20] and in human studies, maternal mental health was affected by oxytocin concentration.[3, 34–36, 45] In addition, maternal psychiatric disorders affect child development and the mother-infant relationship.[3–5] In our study, oxytocin concentration was associated with the presence of specific medical histories. However, it may be that oxytocin concentration could be used to predict the nature of mother-infant relationships. To evaluate this possibility, the JECS questionnaire could be used to correlate the mother's oxytocin concentration with the child's subsequent mental development.

In conclusion, oxytocin concentrations during and after pregnancy were affected by a past history of anxiety disorder and endometriosis. Because oxytocin has a role in social bonding, the mother's medical history may affect the mother-child relationship.

Acknowledgments: We thank Dr. Hiroaki Matsushita, Dr. Hideki Matsui (Okayama University, Okayama, Japan), Dr. Naoki Miyahara and Dr. Susumu Kanzaki (Tottori University, Yonago, Japan) for critical advising this study. The findings and conclusions of this article are solely the responsibility of the authors and do not represent the official views of the Japanese government. This study was supported by JSPS KAKENHI grant number 15K01755, awarded to Y.K and 19K19673, awarded to T.M. We thank Mark Cleasby, PhD, from Edanz Group (www.edanzediting.com/ac) for editing a draft of this manuscript.

The authors declare no conflict of interest.

REFERENCES

1 American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders, 5th ed. Arlington, VA: American Psychiatric Publishing; 2013.
2 Gavin NI, Gaynes BN, Lohr KN, Meltzer-Brody S, Gartlehner G, Swinson T. Perinatal Depression. Obstet Gynecol. 2005;106:1071-83. DOI: 10.1097/01.AOG.0000183597.31630.db, PMID: 16260528
3 Grace SL, Evindar A, Stewart DE. The effect of postpartum depression on child cognitive development and behavior: A review and critical analysis of the literature. Arch Women Ment Health. 2003;6:263-74. DOI: 10.1007/s00737-003-0024-6, PMID: 14628179
4 Brockington I. Postpartum psychiatric disorders. Lancet. 2004;363:303-10. DOI: 10.1016/S0140-6736(03)15390-1, PMID: 14751705
5 Ohoka H, Koide T, Goto S, Murase S, Kanai A, Masuda T, et al. Effects of maternal depressive symptomatology during pregnancy and the postpartum period on infant-mother attachment. Psychiatry Clin Neurosci. 2014;68:631-9. DOI: 10.1111/pcn.12171, PMID: 24521214
6 Anda RF, Felitti VJ, Bremner JD, Walker JD, Whitfield C, Perry BD, et al. The enduring effects of abuse and related adverse experiences in childhood. Eur Arch Psychiatry Clin Neurosci. 2006;256:174-86. DOI: 10.1007/s00406-005-0624-4, PMID: 16311898
7 Nemeroff CB. Paradise Lost: The Neurobiological and Clinical Consequences of Child Abuse and Neglect. Neuron. 2016;89:892-909. DOI: 10.1016/j.neuron.2016.01.019, PMID: 26938439
8 Neumann ID. Alterations in behavioral and neuroendocrine stress coping strategies in pregnant, parturient and lactating rats. Prog Brain Res. 2001;133:143-52. DOI: 10.1016/S0079-6123(01)33011-X, PMID: 11589127
9 Argiolas A, Gessa GL. Central functions of oxytocin. Neurosci Biobehav Rev. 1991;15:217-31. DOI: 10.1016/S0149-7634(05)80002-8, PMID: 1852313
10 Insel TR, Shapiro LE. Oxytocin receptor distribution reflects social organization in monogamous and polygamous voles. Proc Natl Acad Sci USA. 1992;89:5981-5. DOI: 10.1073/pnas.89.13.5981, PMID: 1321430
11 Numan M, Insel TR. The Neurobiology of Parental Behavior. New Jersey: Springer; 2003.
12 Tomizawa K, Iga N, Lu YF, Moriwaki A, Matsushita M, Li ST, et al. Oxytocin improves long-lasting spatial memory during motherhood through MAP kinase cascade. Nat Neurosci. 2003;6:384-90. DOI: 10.1038/nn1023, PMID: 12598900
13 Bielsky IF, Young LJ. Oxytocin, vasopressin, and social recognition in mammals. Peptides. 2004;25:1565-74. DOI: 10.1016/j.peptides.2004.05.019, PMID: 15374658
14 Neumann ID. Brain oxytocin: a key regulator of emotional and social behaviours in both females and males. J Neuroendocrinol. 2008;20:858-65. DOI: 10.1111/j.1365-2826.2008.01726.x, PMID: 18601710
15 Matsushita H, Tomizawa K, Okimoto N, Nishiki T, Ohmori I, Matsui H. Oxytocin mediates the antidepressant effects of mating behavior in male mice. Neurosci Res. 2010;68:151-3. DOI: 10.1016/j.neures.2010.06.007, PMID: 20600375
16 Okimoto N, Bosch OJ, Slattery DA, Pflaum K, Matsushita H, Wei FY, et al. RGS2 mediates the anxiolytic effect of oxytocin. Brain Res. 2012;1453:26-33. DOI: 10.1016/j.brainres.2012.03.012, PMID: 22459044
17 Kosfeld M, Heinrichs M, Zak PJ, Fischbacher U, Fehr E. Oxytocin increases trust in humans. Nature. 2005;435:673-6. DOI: 10.1038/nature03701, PMID: 15931222
18 Hollander E, Bartz J, Chaplin W, Phillips A, Sumner J, Soorya L, et al. Oxytocin increases retention of social cognition in autism. Biol Psychiatry. 2007;61:498-503. DOI: 10.1016/j.biopsych.2006.05.030, PMID: 16904652
19 Guastella AJ, Einfeld SL, Gray KM, Rinehart NJ, Tonge BJ, Lambert TJ, et al. Intranasal oxytocin improves emotion recognition for youth with autism spectrum disorders. Biol Psychiatry. 2010;67:692-4. DOI: 10.1016/j.biopsych.2009.09.020, PMID: 19897177
20 Rich ME, deCárdenas EJ, Lee HJ, Caldwell HK. Impairments in the initiation of maternal behavior in oxytocin receptor knockout mice. PLoS One. 2014;9:e98839. DOI: 10.1371/journal.pone.0098839, PMID: 24892749
21 Kanatani KT, Adachi Y, Sugimoto N, Noma H, Onishi K, Hamazaki K, et al.; Japan Environment & Children's Study Group. Birth cohort study on the effects of desert dust exposure on children's health: protocol of an adjunct study of the Japan Environment & Children's Study. BMJ Open. 2014;4:e004863. DOI: 10.1136/bmjopen-2014-004863, PMID: 24958210

22 Kawamoto T, Nitta H, Murata K, Toda E, Tsukamoto N, Hasegawa M, et al.; Working Group of the Epidemiological Research for Children's Environmental Health. Rationale and study design of the Japan environment and children's study (JECS). BMC Public Health. 2014;14:25. DOI: 10.1186/1471-2458-14-25, PMID: 24410977

23 Kumaresan P, Anandarangam PB, Dianzon W, Vasicka A. Plasma oxytocin levels during human pregnancy and labor as determined by radioimmunoassay. Am J Obstet Gynecol. 1974;119:215-23. DOI: 10.1016/0002-9378(74)90037-4, PMID: 4823390

24 Mizutani S, Hayakawa H, Akiyama H, Sakura H, Yoshino M, Oya M, et al. Simultaneous determinations of plasma oxytocin and serum placental leucine aminopeptidase (P-LAP) during late pregnancy. Clin Biochem. 1982;15:141-5. DOI: 10.1016/S0009-9120(82)90582-3, PMID: 7116622

25 Heinrichs M, Baumgartner T, Kirschbaum C, Ehlert U. Social support and oxytocin interact to suppress cortisol and subjective responses to psychosocial stress. Biol Psychiatry. 2003;54:1389-98. DOI: 10.1016/S0006-3223(03)00465-7, PMID: 14675803

26 Kirsch P, Esslinger C, Chen Q, Mier D, Lis S, Siddhanti S, et al. Oxytocin modulates neural circuitry for social cognition and fear in humans. J Neurosci. 2005;25:11489-93. DOI: 10.1523/JNEUROSCI.3984-05.2005, PMID: 16339042

27 Waldherr M, Neumann ID. Centrally released oxytocin mediates mating-induced anxiolysis in male rats. Proc Natl Acad Sci USA. 2007;104:16681-4. DOI: 10.1073/pnas.0705860104, PMID: 17925443

28 Labuschagne I, Phan KL, Wood A, Angstadt M, Chua P, Heinrichs M, et al. Oxytocin attenuates amygdala reactivity to fear in generalized social anxiety disorder. Neuropsychopharmacology. 2010;35:2403-13. DOI: 10.1038/npp.2010.123, PMID: 20720535

29 Slattery DA, Neumann ID. Oxytocin and Major Depressive Disorder: Experimental and Clinical Evidence for Links to Aetiology and Possible Treatment. Pharmaceuticals (Basel). 2010;3:702-24. DOI: 10.3390/ph3030702, PMID: 27713275

30 Delvoux B, Groothuis P, D'Hooghe T, Kyama C, Dunselman G, Romano A. Increased production of 17beta-estradiol in endometriosis lesions is the result of impaired metabolism. J Clin Endocrinol Metab. 2009;94:876-83. DOI: 10.1210/jc.2008-2218, PMID: 19088158

31 Kimura T, Saji F, Nishimori K, Ogita K, Nakamura H, Koyama M, et al. Molecular regulation of the oxytocin receptor in peripheral organs. J Mol Endocrinol. 2003;30:109-15. DOI: 10.1677/jme.0.0300109, PMID: 12683935

32 Fuchs AR, Fuchs F, Husslein P, Soloff MS. Oxytocin receptors in the human uterus during pregnancy and parturition. Am J Obstet Gynecol. 1984;150:734-41. DOI: 10.1016/0002-9378(84)90677-X, PMID: 6093538

33 Huang M, Li X, Guo P, Yu Z, Xu Y, Wei Z. The abnormal expression of oxytocin receptors in the uterine junctional zone in women with endometriosis. Reprod Biol Endocrinol. 2017;15:1. DOI: 10.1186/s12958-016-0220-7, PMID: 28049501

34 Jonas W, Mileva-Seitz V, Girard AW, Bisceglia R, Kennedy JL, Sokolowski M, et al.; MAVAN Research Team. Genetic variation in oxytocin rs2740210 and early adversity associated with postpartum depression and breastfeeding duration. Genes Brain Behav. 2013;12:n/a. DOI: 10.1111/gbb.12069, PMID: 23941164

35 Clarici A, Pellizzoni S, Guaschino S, Alberico S, Bembich S, Giuliani R, et al. Intranasal adminsitration of oxytocin in postnatal depression: implications for psychodynamic psychotherapy from a randomized double-blind pilot study. Front Psychol. 2015;06:426. DOI: 10.3389/fpsyg.2015.00426, PMID: 25941501

36 Jobst A, Padberg F, Mauer MC, Daltrozzo T, Bauriedl-Schmidt C, Sabass L, et al. Lower Oxytocin Plasma Levels in Borderline Patients with Unresolved Attachment Representations. Front Hum Neurosci. 2016;10:125. DOI: 10.3389/fnhum.2016.00125, PMID: 27064696

37 Mah BL, Van IJzendoorn MH, Smith R, Bakermans-Kranenburg MJ. Oxytocin in postnatally depressed mothers: its influence on mood and expressed emotion. Prog Neuropsychopharmacol Biol Psychiatry. 2013;40:267-72. DOI: 10.1016/j.pnpbp.2012.10.005, PMID: 23085508

38 Muchanga SMJ, Yasumitsu-Lovell K, Eitoku M, Mbelambela EP, Ninomiya H, Komori K, et al.; Japan Environment and Children's Study Group. Preconception gynecological risk factors of postpartum depression among Japanese women: The Japan Environment and Children's Study (JECS). J Affect Disord. 2017;217:34-41. DOI: 10.1016/j.jad.2017.03.049, PMID: 28365479

39 Amico JA, Tenicela R, Johnston J, Robinson AG. A time-dependent peak of oxytocin exists in cerebrospinal fluid but not in plasma of humans. J Clin Endocrinol Metab. 1983;57:947-51. DOI: 10.1210/jcem-57-5-947, PMID: 6619269

40 Lindow SW, Newham A, Hendricks MS, Thompson JW, Van Der Spuy ZM. The 24-hour rhythm of oxytocin and B-endorphin secretion in human pregnancy. Horumon To Rinsho. 1996;45:443-6. DOI: 10.1046/j.1365-2265.1996.8290840.x, PMID: 8959083

41 Gard JW, Alexander JM, Bawdon RE, Albrecht JT. Oxytocin preparation stability in several common obstetric intravenous solutions. Am J Obstet Gynecol. 2002;186:496-8. DOI: 10.1067/mob.2002.121104, PMID: 11904613

42 Kumar V, Madabushi R, Derendorf H, Boothby LA, Breland BD, Hatton RC, et al. Development and Validation of an HPLC Method for Oxytocin in Ringer's Lactate and its Application in Stability Analysis. J Liq Chromatogr Relat Technol. 2006;29:2353-65. DOI: 10.1080/10826070600864742

43 Trissel LA, Zhang Y, Douglas K, Kastango E. Extended stability of oxytocin in common infusion solutions. Int J Pharm Compd. 2006;10:156-8. PMID: 23974190

44 Zhang G, Zhang Y, Fast DM, Lin Z, Steenwyk R. Ultra sensitive quantitation of endogenous oxytocin in rat and human plasma using a two-dimensional liquid chromatography–tandem mass spectrometry assay. Anal Biochem. 2011;416:45-52. DOI: 10.1016/j.ab.2011.04.041, PMID: 21609710

45 Romano A, Tempesta B, Micioni Di Bonaventura MV, Gaetani S. From Autism to Eating Disorders and More: The Role of Oxytocin in Neuropsychiatric Disorders. Front Neurosci. 2016;9:497. DOI: 10.3389/fnins.2015.00497, PMID: 26793046