Oxytocin during pregnancy and early postpartum: Individual patterns and maternal–fetal attachment

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1. Introduction

The role of the nanopeptide hormone oxytocin (OT) has been extensively studied in relation to social affiliation in rodents
[4,7,21,44] and to a somewhat lesser extent in humans as well
[5,19,44]. The importance of OT for the initiation of maternal behavior was first noted by Pedersen and Prange, Jr. [34]. More recently, OT has been found to be instrumental in the maintenance of specific elements of maternal behavior such as pup-licking and upright nursing postures in rat dams [33], whereas OT antagonists decrease pup licking and upright posturing over pups, maternal bonding behaviors that have been linked to reduced fear responses under conditions of novelty [9]. Differences persisted into adulthood, affecting both maternal behaviors and OT receptor binding [33].

Despite the fact that much research has illuminated the role of OT in the initiation of maternal behaviors in mammals, little is known about whether or not this function of OT is present in human mothers. In one relevant paper, Fries et al. [15] studied the oxytocin levels of adopted children with backgrounds of early neglect. Subjects had resided, since early infancy, in orphanages for an average of 17 months and had been fostered for three years. Subjects had similar basal urine OT levels as controls, as assessed by averaging 12-h overnight urine collections for four separate mornings. However, in striking contrast to controls, the adopted children showed decisively less increase in oxytocin levels after physical contact with their caregivers. Since subjects had already resided in their foster homes for 3 years (range 10–48 months) at the time of testing, the authors concluded that the lack of...
early oxytocin-related maternal bonding had a long-range effect on these children, an effect apparently resistant to subsequent nurturing.

Human maternal behaviors, like those of other mammals, begin to appear during pregnancy and are likely to have a hormonal basis [35]. OT is likely to play a role in the development of the mother’s bond to her fetus. Such bonding behaviors include maternal self-caring in ways which could influence fetal development [11]. Fetal development may also be affected at the site of the maternal–infant hormonal interface [6]. From the infant’s perspective, it is still not known whether direct or indirect links exist between maternal and fetal OT. Studies in both baboons [31] and humans [25,32,41] report mixed results. Should maternal OT enter fetuses peripherally it could pass the blood–brain barrier and influence brain ontogeny. In adult human subjects, both peripheral injection [27] and nasal administration [19] of OT have produced central effects. Systematic research on the effects of maternal OT levels upon the development of the fetus and neonate would benefit from the establishment of characteristics of OT over the perinatal period including range of expected values and patterns over time. Manipulation of OT levels during early neonatal development may influence the expression of adult behavior and physiology [3]. For example, injections of 3 μg of OT or an OT antagonist in 24 h old prairie vole pups significantly affected elements of adult reproductive behavior and success [12]. In humans, OT has been implicated in the etiology of autism [20], and lower levels of OT have been reported in both autistic people [28] and patients with major depression [37]. Thus, elucidation of maternal OT levels and patterns in normative community samples could allow for the study of how deviations from such values may impact on bonding and other maternal and offspring parameters.

To date, few studies have examined perinatal OT levels in humans and thus, a clear characterization of OT levels across pregnancy and the postpartum is not yet available. Dawood et al. [13] studied OT in maternal circulation during pregnancy and reported a wide scatter of between-subjects values throughout pregnancy. The data showed a positive correlation between OT and week of pregnancy with values generally higher in the second half of gestation, ranging between 17.9 pg/ml (±15.2) and 74.2 (±14.2) pg/ml. Leake et al. [26] attempted to establish OT levels across pregnancy and found no differences between male and nonpregnant female groups and pregnant females except when the latter entered contractions. The authors reported that OT levels remain low over pregnancy with mean between-subject values of 2.6 ± 2.2 pg/ml. De Geest et al. [14] concluded that while a wide range of values existed throughout pregnancy, a rising pattern across pregnancy was common, with mean values across pregnancy ranging from 94 pg/ml at 12 weeks to 272 pg/ml at 40 weeks. Despite noticing some individual differences and fluctuations, researchers throughout the 1980s alternatively described oxytocin as remaining at basal levels until labor or as gradually rising across the course of pregnancy (for review see [16]). In the 1990s, van der Post et al. [42] reported no rise in OT above basal levels up to 253 days gestation, while Silber et al. [38] reported significant increases in OT above controls when compared at 36 weeks gestation. Altemus et al. [1] also reported higher OT in pregnant women compared to nonpregnant controls at 38–40 weeks pregnancy. Many researchers (see e.g. [10,25]) noted the inconsistency of findings and variously explained them as due to differences in antiseras, extraction techniques, or other problems related to the measuring method.

The goal of the present study was to present a comprehensive description of OT across pregnancy and the first postpartum month, and to examine the relationship between these OT values and maternal-fetal bonding. Healthy pregnant women were tested at three time points and OT was measured by enzyme immunoassay (EIA; [45,46]).

We sought to describe characteristic values as related to week in pregnancy, address whether OT levels rise or fall across the time-period and describe patterns for OT levels in individual women. The relations between levels and patterns of OT and the mother’s self-reported bonding with her fetus during the third trimester of pregnancy were also tested. These data present the first longitudinal assessment of OT patterns across pregnancy and the postpartum in healthy women, using EIA.

2. Materials and methods

2.1. Participants

Original participants in this longitudinal study were 78 pregnant women in the first trimester of pregnancy recruited from a pool of expecting mothers receiving their prenatal care in the nation-wide well-baby clinic service. Mean age was 28.2 years (S.E.M. = .62, range of 18.4–43.2 years) and 49% were expecting their first child. Fifty three percent of the women had high-school education, 23% had some higher education and 24% had at least one college degree. Thirty-three percent of the women did not work outside the home, 14% worked outside the home part-time (<1/3 position) and 53% worked in a more extensive position (33–100%). Women were excluded from the study if they were less than 18 years old, not in a committed relationship with the genetic father, or used psychiatric medication or hormonal treatment. All women were in good general health as assessed by nurses. Women gave birth at a mean of 39.46 weeks (S.E.M. = .26 with a range of 32–42 weeks). The study was approved by the IRB and all women signed an informed consent form prior to participating in the study.

Twelve women did not continue past Time-I (early pregnancy): six due to abortions and six due to technical reasons. Two additional women did not drop out but had missing data. Therefore, 66 women remained in the study. Data for Time-II (early third trimester) exist for only 64 of them. Four more women did not continue after Time-II, 2 due to premature births and 2 due to technical problems, leaving 62 women who completed all three assessments.

2.2. Study design

Blood was sampled at three separate time points: Time-I – early pregnancy (M = 10 weeks, S.E.M. = .32, range = 6–16); Time-II – early third trimester (M = 27.35 weeks, S.E.M. = .27, range = 22.43–32.72); and Time-III – early postpartum (M = 1.84
weeks, S.E.M. = .11, range = .57–4.14). At Time II, a prenatal bonding self-report questionnaire (the MFAS) was completed.

2.3. **OT sampling and assay**

Blood was sampled between 08:00 and 10:00 AM by a single draw of 5 ml of blood from antecubital veins. Blood was drawn into chilled vacutainer tubes containing lithium heparin that were injected with 200 µl of Trasylol (aprotinin) 500,000 KIU (Bayer, Germany). OT samples were kept ice-chilled for up to 4 h before being centrifuged at 4 °C at 1800G for 15 min. Supernatants were collected and stored at −70 °C until assayed.

Determination of OT was performed using a 96 plate commercial OT EIA kit (R & D Systems, Minneapolis, USA). This EIA is a competitive immunoassay for the quantitative determination of OT and considered to be a very sensitive assay [23]. The kit recognizes exclusively OT and not other nanopeptides such as arginine vasopressin (AVP) and somatostatin. The validity of this method was confirmed by Kramer et al. [23], who concluded that “the EIA is valid and can be used to reliably measure plasma OT concentrations”, listing in addition a number of advantages this method has over available RIA kits [23, p. 1198]. More recently, Carter et al. [8] ran plasma samples through high pressure liquid chromatography (HPLC) prior to immunoassay, splitting OT samples and comparing EIA with RIA. The EIA was shown to be highly specific for OT in plasma while the comparison RIA was less specific.

Measurements were performed in duplicate. Each subject’s samples were assayed for all time-periods of participation in the same batch to avoid differences due to inter-assay variance. Samples were diluted 1:5 in the assay buffer and treated according to the instructions of the commercial kit. At the final step, the optical density of the samples and standards was measured in wavelengths of 405 and 590. The concentrations of samples were calculated by using Matlab-6 according to the relevant standard curve having the range of 3.9–1000 pg/ml. For each plate a separate standard curve was constructed.

Measurements were calculated for the total range as for the main range of OT values. OT average values, presented as means and medians, range between .80 and .87 [29]. Cronbach’s alpha for our study was .86.

2.5. **Data analysis**

Pearson product–moment correlations were employed to examine correlations between OT levels at the different time points. Two-way analysis of variance (ANOVA) with repeated measures was employed to test for significant differences in patterns of OT levels over time, between five different sub-groups with distinct patterns. Pair-wise comparisons of sub-groups followed, by similar ANOVAs. Post-hoc one-way ANOVA with repeated measures were next performed separately in each sub-group, to search for significant trends. Difference between specific time-points was further examined by paired t-tests, within each sub-group. One-way ANOVA was also used to compare the reported levels of maternal–fetal bonding in women with different profiles/typologies of OT, in the last portion of the data analysis.

3. **Results**

3.1. **OT distribution**

Values of OT over each of the three time periods were examined. We found that the grand range for OT was 45.0–3448.7 pg/ml (Table 1, Fig. 1). However, the range of values was not continuous. While most women’s values fell within the range of 45–546 pg/ml (Fig. 1), five women consistently had values ranging between 846 pg/ml and 3648 pg/ml.

As seen in Fig. 1 the distribution of OT levels showed a semi-bell shape form for all examined periods, ranging between 45 pg/ml and 250 pg/ml before tailing off to values up to 400 pg/ml for Time-I (Fig. 1, Time-I). Time-II has a range of 60–250 pg/ml before tailing off to levels up to 450 pg/ml (Fig. 1, Time-II). Time-III ranges to a nominally higher value than the others, 50–350 pg/ml, before tailing off to the highest values for all distributions, 550 pg/ml. (Fig. 1, Time-III).

Even though all distributions are outwardly similar there seems to be a slight upward shift after Time-I. Time-I shows a wide peak in the range of 150–200 pg/ml, while both Time-II and Time-III have peak values of 200.

Due to the presence of a non-continuous group of high levels, we further analyzed the statistical properties of OT values. OT average values, presented as means and medians, were calculated for the total range as for the main range of OT values (Table 1). The average values do not appear to be representative while the medians reflect OT levels at each time point. As can be seen (Table 1) the average values changed dramatically when the higher values were excluded, and average values became of similar range to that of the medians. This manipulation did not produce clear-cut changes in median values.

Correlations were found between all time points: r = .93, p < .001, between Time I and Time II; r = .96, p < .001, between Time II and Time III; and r = .92, p < .001, between Time I and Time III. In summary, a very stable picture arises for the course of OT across pregnancy and the postpartum.

2.4. **The maternal–fetal attachment scale (MFAS)**

The MFAS [11] is a 24 item scale with reported reliability [29]. Responders rate the degree to which statements reflect their personal opinion. Examples of statements are: “I speak to my baby in my womb,” and “I imagine myself nursing my baby.” Responses range from “very much so” (scored 1) to “entirely not so” (scored 5). The numerical equivalents of each response are summed and averaged to arrive at a total score. Five subscale scores (role-taking, giving, attributes, interaction and differentiation) are arrived at by the same method. Studies have found Cronbach’s alpha coefficients for the MFAS to
3.2. OT scatter-plots by week

In the current study we observed a small shift in the distribution toward higher values of OT in Time-II and Time-III. Therefore, in order to discern whether this pattern is correlated with day of sampling, we plotted OT values by week of gestation and postpartum (Fig. 2). Each of three distinct ‘clouds’ of values appears consecutively with the range of weeks for its sampling time-period. It is apparent that there is no correlation whatsoever between day of pregnancy or day postpartum and OT levels. Interestingly, the higher OT levels are grouped in week 8, for Time-I and in the 4th postpartum week. Unfortunately, the number of women at each week is too small for further analysis of these observations.

3.3. OT typologies

In addition to the global analysis based on data from entire distributions, we chose to analyze data for individual women. The results of this analysis appear in Fig. 3 A–E. According to the extant literature, OT levels are expected either to rise or to remain at basal levels throughout pregnancy. Until we performed an individual subject analysis of our own data, our mean results appeared to present a uniform trend for all women. However, the examination of individual patterns over time showed five distinct trends (Fig. 3). Two-way ANOVA with
repeated measures showed that these patterns were significantly different \( F(8,74) = 11.17, p < 0.001 \), for the five subgroups \( \times \) three times interaction factor). Interestingly, these patterns appear in near mirror images. ANOVA analyses showed that all five patterns presented (A–E) are significantly distinct from each other, as determined by two-way ANOVA with repeated measures. (A) Stable OT levels across pregnancy and the postpartum. (B) OT levels rose from Time-I to Time-III. (C) OT levels decreased between Time-I and Time-III. (D) OT levels rose from Time-I to Time-II and dropped from Time-II to Time-III. (E) OT levels dropped between Time-I and Time-II and then increased from Time-II to Time-III. For (A–E): \( N = 14, 6, 7, 8 \) and 8, respectively. The flat typology shows, for presentation considerations, only women with OT values less than 550 pg/ml. All women with OT values above 550 pg/ml had flat patterns.

Fig. 3 – (A–E): OT Typologies. Typologies are comprised of values for those women for whom we had data for all three Times. The points charted represent the group average for each of the three Time points for each typology. All five patterns presented (A–E) are significantly distinct from each other, as determined by two-way ANOVA with repeated measures. (A) Stable OT levels across pregnancy and the postpartum. (B) OT levels rose from Time-I to Time-III. (C) OT levels decreased between Time-I and Time-III. (D) OT levels rose from Time-I to Time-II and dropped from Time-II to Time-III. (E) OT levels dropped between Time-I and Time-II and then increased from Time-II to Time-III. For (A–E): \( N = 14, 6, 7, 8 \) and 8, respectively. The flat typology shows, for presentation considerations, only women with OT values less than 550 pg/ml. All women with OT values above 550 pg/ml had flat patterns.
occurred to relatively moderate degrees (between 10% and 60%).

3.4. Prenatal bonding as a function of a rise in OT from early to late-mid pregnancy

Mean scores for total MFAS and all subscales, as determined by the scale’s developer, MS Cranley, are nearly identical to those found in the present study (Table 2).

Since the five typologies showed OT as either rising, dropping or remaining flat between time-periods, we were interested in exploring whether these different patterns were correlated with levels of reported prenatal bonding. One-way ANOVA with post-hoc Duncan tests was used to compare women with increasing (N = 13), decreasing (N = 13) and flat (N = 19) patterns. These tests (data not shown) showed that the group with increasing OT levels between Time-I and Time-II exhibited significantly higher scores on the total MFAS as well as on two of its subscales, differentiation and attributing to fetus (F(2,42) = 8.67, 9.56 and 8.00, respectively, all p < 0.001), compared to the other groups. OT levels measured at Times-I and II did not correlate significantly with either the total MFAS score or any of its five subscales. Thus, it appears that the relationship between the rising trend of OT between Times-I and -II and bonding was independent of both Time-I and -II OT levels.

4. Discussion

4.1. Wide ranges and characteristic individual levels

The wide range of pregnancy-related OT values found in this study has been noted previously and seems to be characteristic. Dawood et al. [13] reported a ‘wide scatter’ of values across pregnancy. In early pregnancy they reported a 60-fold range of 1.5 pg/ml to 90 pg/ml. Similarly, De Geest et al. [14] reported ‘wide differences’ in values throughout pregnancy. By comparison, and for the same period of early pregnancy, their lowest value was 10 µU/ml and their highest value was 130 µU/ml, a more than 10-fold difference. The present study also reports a large range of values. The reasons for this broad range across individuals are unknown.

Despite the reported wide overall range of values, the question arises whether certain magnitudes of values are characteristic of individual women. Vasicka et al. [43], reporting on within-subject data, remarked on the consistent production of low or high levels of OT throughout pregnancy by their patients. Uvnas-Moberg et al. [40] found that within-subject breastfeeding OT levels, sampled twice (at 4 days and again at 3-4 months) postpartum correlated significantly. In accordance, our study further expands on this pattern, by reporting strong and significant rank-order correlations for within-subject OT levels for all three time-periods, covering a 6 month epoch and spanning two distinct phases, pregnancy and the early postpartum.

4.2. Levels of OT in the perinatal period

There are no established absolute norms for OT values across pregnancy and the postpartum. It is therefore not presently possible to say what normal or expected values are. It would not seem appropriate to compare our ELISA data with previous data assayed with RIA. The number of studies using ELISA is still limited. In one animal study researchers found that their observed OT concentrations were substantially higher than previously reported for RIA. Those authors noted that systematic comparison of the two assay types shows that ELISAs tend to yield higher values. In two non-pregnancy studies with humans, Zak et al. [45,46] reported values, assayed by ELISA, ranging between 25 pg/ml and 450 pg/ml. Their data set also contained values up to nearly 1000 pg/ml as well as two subjects with values of around 1100 pg/ml and one subject with two values around 3300 pg/ml (personal communication). Our levels are the highest published using ELISA. We believe that the highest-range of values found by Zak et al. are, like ours, valid. We base this on our findings with five women, all of whom had two or more values in the 1000–3600 range at large time intervals, levels which parallel Zak et al.'s own single time-period outliers. The values appearing in this study are also the first published for a longitudinal pregnancy study using ELISA.

The ‘high-range’ OT values found in our study are both interesting and intriguing. Interesting because they represent values for nearly 10% of our subjects, subjects who came from a nonpathological and community-based population. Intriguing, because despite the absence of established norms by which to definitively determine what should be considered high-range values, they are, even for the present study’s range of data ‘outliers’. Previous studies have attempted to interpret the significance of their own highest values. Both physiological and psychological interpretations have been made. Vasicka et al. [43], for example proposed that high OT levels during pregnancy might be related to disease states such as toxemia, but did not pursue this possibility systematically. Turner et al. [39] found that their higher OT levels were associated with greater interpersonal stress. This later finding would seem to run counter to the main thrust of OT literature, as it pertains to emotions, personality and mood. Nevertheless, a more complex oxytocin-stress relationship may yet be uncovered, as appears to be the case for cortisol and maternal behavior [24].
4.3. Typologies

The question of whether or not OT levels increase or not over pregnancy is unanswered by studies using RIA methods. Several authors have claimed no increase [26,42] while others claim that an increase takes place [38,43]. Most preclinical studies show little or no increase in hypothalamic production of OT during pregnancy [36]. However, this does not in any way inform our question. It must be emphasized that, since we are dealing here with human research, we are referring specifically to peripheral OT levels. Peripheral levels of oxytocin may not reflect central levels [1], despite a degree of synchrony [22], as they originate from different hypothalamic cells [39]. Furthermore, even central production rates for that oxytocin which is eventually to be transported to the periphery, are not necessarily mirrored by peripheral levels. This is due, in part, to the fact that a certain amount of peripheral OT originates from many sites in the periphery itself, including the uterus [2] and the heart [17].

The present study described five typologies (Fig. 3A–E) in place of the previous rise and no-rise depictions. Detailed examination of data from many previous studies shows that trends are not strictly linear and some authors even noted the existence of ‘fluctuations’ (see [13], p. 431). It is possible that such typologies existed in previous studies, but the use of small within-subject cohorts or large between-subject cohorts may have precluded noticing such patterns. We note that dramatically opposite response patterns in hormonal levels have been reported in other neuroendocrine systems, e.g. a considerable numbers of people show hypocortisolism in response to stress [18].

4.4. Maternal–fetal bonding and increasing OT levels over pregnancy

Our sample reported mean scores for total MFAS and all subscales that were very similar to those reported for a large US sample by the scale’s developer (Table 2). This is interesting since testing took place nearly 25 years apart in two different cultures (USA and Israeli) and for different magnitudes of group sizes (346 versus 64). This suggests a cross-cultural validity for the type of comparison we made. Furthermore, it would appear that prenatal bonding has a well-conserved similarity in the factor structure of prenatal attachment. Thus, Cranley’s scale, despite its critiques (see [30]) appears to have validity for the type of comparison we made. Furthermore, it would seem that prenatal bonding has a well-conserved structure of content that is not strongly influenced by culture or era.

This is the first study to examine the relationship between plasma OT and reported maternal–fetal attachment. In contrast to our expectations, levels of reported bonding were not significantly correlated with OT levels in pregnancy, assessed earlier (first trimester) or concomitantly (third trimester). However, participants whose OT levels rose between early to late-mid pregnancy scored higher on prenatal bonding, compared to the other women. Thus, dynamically increasing levels of OT and not static, however large, levels of OT were associated with increased prenatal bonding. It can be noticed from the typology figures (Fig. 3) that the rising OT levels between Time-I and -II which is important in creating the association between them and better prenatal bonding.

5. Conclusions

A longitudinal description of OT levels throughout pregnancy and the early postpartum has been presented. This description is based on an EIA assay of plasma, within-subjects, from a community sample. The present study suggests that perinatal OT patterns are more varied than previously thought. This finding needs to be replicated using more sampling times to establish its validity. OT levels higher than previously reported raise the important need for establishing an accepted range of values in order to allow for the evaluation and comparison of reported results. Increase in OT from early to late pregnancy correlated with higher maternal–fetal bonding.

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