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The endogenous oxytocin after manipulative osteopathic treatment in full-term pregnant women

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Abstract. – OBJECTIVE: The aim of this study is to assess whether the touch of osteopathic manipulative treatment (OMT) can affect the endogenous production of oxytocin in full-term pregnant women and the assessment of well-being following the treatment.

PATIENTS AND METHODS: In this study have been enrolled 57 pregnant women at full-term pregnancy (37th-41st week) for evaluation of the concentration of salivary oxytocin 2 minutes before and 2 minutes after a single session of OMT by an osteopath lasting for 30 minutes. Pre-OMT and post-OMT saliva samples were collected with the use of Salivette® salivary swabs. 7 salivary swabs were excluded from the analysis. 50 samples were analyzed with an appropriate ELISA kit.

RESULTS: The mean OT salivary concentration pre-OMT was 89.98±16.39, and post-OMT was 100.60±19.13 tends to increase with p=0.0000051. In multivariate analysis, two subgroups show interesting data in the mean difference in OT salivary concentration post-OMT: women with painful contractions (p=0.06) and women under 35 years (p=0.09).

CONCLUSIONS: The results of this study demonstrate that the effectiveness of OMT-increasing endogenous oxytocin is statistically significant in full-term pregnant women. The sensation of well-being found in most women indicates that there has been a predominantly central rather than peripheral oxytocin release after OMT.

Key Words:
Osteopathic manipulative treatment (OMT), Oxytocin, Pregnant women, Synthetic Oxytocin, Full-term pregnant women.

Introduction

Oxytocin (OT) is a neuropeptide, synthesized above all by magnocellular neurons of the paraventricular and supraoptic hypothalamus nuclei, and with oxytocinergic fibers reach different areas of the nervous system that performs its neurogenic effects. Some studies[1-4] have shown that social, affective touch, and/or massage treatments reduce anxiety, stress, heart rate, blood pressure, pain, and other clinical conditions[5,6]. Therapeutic shoulders, neck, and back massages can increase plasma OT concentrations and consequently reduce the adrenocorticotropin hormone, which is responsible for an anxiolytic effect[7,8].

Osteopathic manipulative treatment (OMT) is a manual therapy used to diagnose and treat somatic dysfunction, including pregnant women[9]. OMT has been studied in obstetric and gynecological patients, and some studies have shown improvements in low back pain and pregnancy outcomes in terms of pain relief and functional optimization when women were given OMT during the prenatal period without adverse effects[10,11]. Other studies[12] have suggested that OMT has an anti-inflammatory effect, as demonstrated by the reduction of serum level of TNF-α and influences the hormonal profile, promoting a change in the concentration of endocannabinoids and serotonin, arginine, vasopressin, cortisol, and OT.

When synthetic oxytocin (SynOT) is administered intravenously, only a small portion reaches

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the cerebrospinal fluid (only 0.001%) because the blood-brain barrier is poorly permeable to SynOT[13]. For this reason, SynOT with this route of administration causes numerous peripheral effects but few central effects[14]. On the contrary, the stimulation of the production of endogenous OT allows it to determine its central effects (reduction of pain perception; maternal-infant bonding and social affiliation, fear, memory, maternal behaviors learning, anxiety, eating behavior, and pain perception), with less relevance to its peripheral effects. Unfortunately, there are few studies concerning the effects of SynOT on the maternal neuro-hormonal system and, consequently, on the mother-child dyad[15].

To our knowledge, we propose for the first time a study to evaluate whether OMT can modify the endogenous production of OT in pregnant women at term, conferring the neuroendocrine advantages by increasing endogenous OT without triggering labor and the assessment of well-being following the treatment.

Patients and Methods

57 women at term of pregnancy were enrolled in this study, between February 16th, 2022, to March 7th, 2022, for evaluation of the concentration of salivary OT before and after OMT. This study is conducted by the regulatory standards of Good Clinical Practice and the Declaration of Helsinki (1996) and was approved by the Internal Review Board of Campus Bio-Medico of Rome (Prot. N. 66/2022). This research received no specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Inclusion criteria were: full-term pregnancy (37th-41st week), age ≥ 18 years, single fetus, in the longitudinal situation and cephalic presentation, and absence of manifest pathologies.

Exclusion criteria: preterm pregnancy (<37th week), age < 18 years, more than one fetus, non-cephalic fetus presentation, pathological pregnancy, active labor.

To avoid bias on contractile activity derived by preterm birth and for the safety of the patients in the study, we chose to perform the study on a population of full-term pregnant women.

Figure 1 reports the flowchart of the research. The OMT was administered to 57 full-term pregnancy patients.

Upon signing informed consent, the women were subjected to OMT by an osteopath of the ISO (Istituto Superiore di Osteopatia) in Milan.

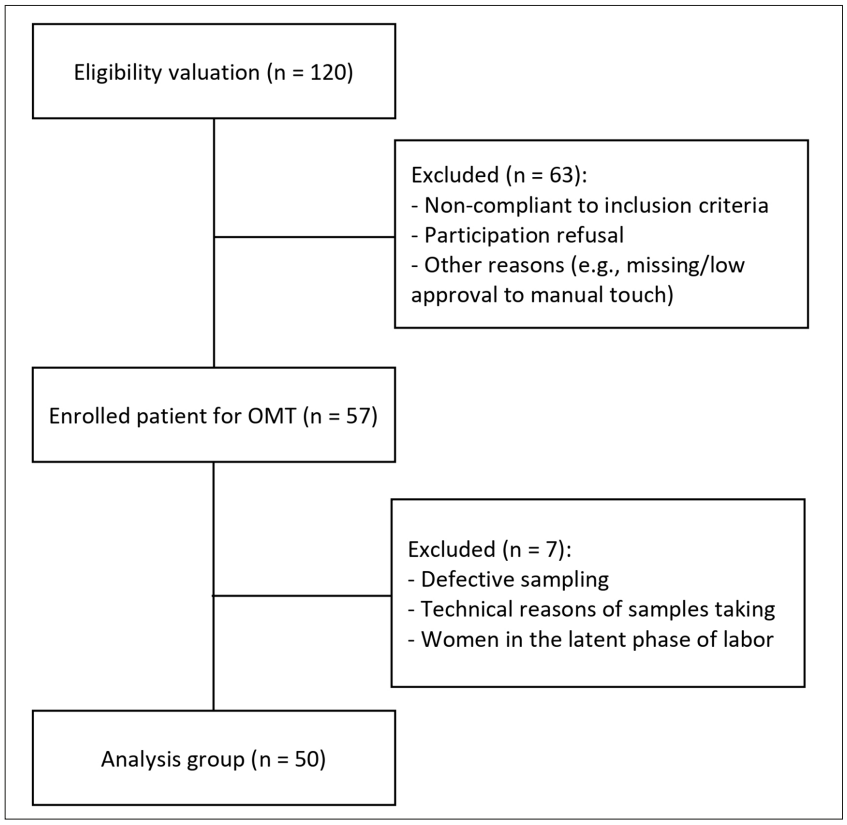

Figure 1. Participant recruitment flowchart.

Before proceeding with the treatment, patients were invited to provide their personal and anamnestic data: age, weight, and BMI at term, height, pathologies, previous surgeries, parity, modalities of any previous childbirth, assisted reproductive technologies, and presence of reported painful contractions. Two minutes before the OMT, the women were subjected to salivary swabs for salivary OT assay using a commercial 100-plate ELISA kit (ab133050) by the same operator inside the medical room (with a controlled temperature of 23°C). In the same room, OMT consisted of a single session with a total duration of 30 minutes that included: Myofascial-release sub-occipital, Myofascial-release scaleni, Myofascial-release upper chest tight and trapezoids, Cranial-technique on the large cerebral scythe, Myofascial technique on the central tendon, Cathie technique on the psoas, external shutter Muscle Energy Technique, C0-C1 AT (Articulatory Technique), C3-C5 AT, C7-D1 AT, AT from D5-L3, sacred AT, coxo-femoral AT. Two minutes after the end of OMT treatment, in the same room, the women were subjected to salivary swab for salivary OT assay using a commercial 100-plate ELISA kit (ab133050). We used Salivette® (STARSTEDT AG & Co. KG, Nümbrecht, Germany) as salivary swabs for the assessment of salivary oxytocin concentration. After the second salivary swab, the osteopath administered an anonymous patient satisfaction questionnaire structured into 10 questions.

Salivary samples (0.3-0.8 ml) were treated according to the manufacturer's instructions, collected by an absorbent roller held in the mouth inside the cheek for 2 minutes without chewing, inserted and closed in plastic microtubes (1.5 ml) and immediately centrifuged at 1000×g at 4°C for 2 minutes. Finally, the samples were stored at -20°C in the freezer until they were delivered and analyzed by the analysis laboratory. All data of OT salivary concentration pre- and post-OMT were collected in an Excel sheet (software version 16.0).

Statistical Analysis

The resulting *p*-value was evaluated as an index of significance to the parameter $\alpha=0.05$. The significance of the data collected was then measured by regression analysis between A (independent variable) and B (dependent variable). The regression statistic must be interpreted based on a key prerogative of OT due to its wide variability. A second analysis with the Student's *t*-test was conducted on the pre- and post-OMT mean OT salivary concentration variation. In particular, the null hypothesis to reject is that the pre- and post-OMT mean OT salivary concentration change is 0. To verify the validity of this hypothesis, the analysis was carried out with the calculation of the change in OT salivary concentration of pre- and post-OMT. Following the significance analysis of the entire sample, a multivariate analysis was conducted by grouping patients into different clusters based on information acquired during the pre-OMT history.

The statistical analysis was conducted with the software Stata® (version 16.1). 7 samples (12.2%) were excluded from the analysis due to incorrect sampling or because they were related to the latent phase of labor. The final dataset consists of 50 pregnant women at the end of pregnancy. The minimum number of patients required to reach statistical significance in this study was calculated based on 95% power to detect a significant difference in the salivary OT level after OMT at a 5% significant level of 34 patients. To protect patient privacy, as already mentioned, an information sheet and an informed consent form were used to enroll in the study and manage sensitive data.

Results

The demographic and clinical features of the cohort are collected (Table I). The resulting data from this paper are as follows. The mean OT salivary concentration pre-OMT(A) was 89.98±16.39, and post-OMT(B) was 100.60±19.13, which tends to increase. The result of linear regression with the relationship of dependence between the two variables is described by the function: $B=25.021+0.84\cdot A$. This function is characterized by a not particularly high R^2 (0.518) value and a Standard Error=13.42; nevertheless, to be interpreted considering the inherent characteristic of OT salivary concentration, namely its high variability, the t-angular coefficient test shows a significant *p*-value=3.87^{-9} (p=0.0000051).

A second analysis using the Student's *t*-test on the data of the mean salivary concentration and variation pre-and post-OMT obtained a *p*-value=5.74^{-7} (p=0.0000049). It is possible to reject the null hypothesis and infer that the mean OT salivary concentration change due to the OMT is significantly higher than 0. The other main descriptive statistics pre-OMT(A) and post-OMT(B) of OT salivary concentration values were collected (Table II). The result of regres-

Table I. The demographic and clinical features of the cohort.

	Number of patients (n = 50)	N, (%)
Age, years (mean ± SD)	34.71 ± 5.13	-
Weight, Kg (mean ± SD)	73.8 ± 11.6	-
Height, cm (mean ± SD)	165 cm ± 6.4	-
Maternal BMI (mean ± SD)	25.67 ± 3.76	-
Gestational age	38.98 ± 1.30	-
Parity, n (%)	Nulliparous	29 (58)
	Multiparous	21 (42)
Maternal ethnicity, n (%)	Italian	44 (88)
	Foreign	6 (12)
Pathologies n, (%)	Yes	15 (30)
	No	35 (70)
Previous surgeries n, (%)	Yes	27 (54)
	No	23 (46)
Previous childbirth, n, (%)	Vaginal birth	17 (34)
	Cesarean Section	4 (8)
Assisted by reproductive technologies n, (%)	Yes	5 (10)
	No	45 (90)
Allergy	Yes	15 (30)
	No	35 (70)
Presence of contractions referred to as painful	Yes	15 (30)
	No	35 (70)

sion analysis suggests that in the presence of OMT between two OT salivary concentration detections, the second one (in this case B) is, on average, higher than the first (A). However, this difference is due to high volatility and decreases as pre-OMT salivary concentration of OT values increases. The concentration of OT can widely vary from individual to individual, thus entailing the possibility that the significance obtained may be the result of chance, is instead attenuated by the presence of a particularly low *p*-value, and consequently more indicative.

The distribution of mean post-OMT oxytocin concentration was increased between 40-50 pg/mL in 2 patients, 30-40 pg/mL in 3 patients, 20-30 pg/mL in 7 patients, 10-20 pg/mL in 12 patients and 0-10 pg/mL in 14 patients. The mean post-OMT oxytocin concentration was decreased between 0-10 pg/mL in 10 patients and 10-20 pg/mL in 2 patients. Negative variations are generally smaller than positive ones.

In multivariate analysis, two subgroups show interesting data in the mean difference OT salivary concentration post-OMT: women with painful contractions (*p*-value=0.06) and women under 35 years (*p*-value=0.09). The other results of the multivariate analysis are collected (Table III). The result of an anonymous questionnaire on patient satisfaction, structured in 10 questions, reports a high approval rating of OMT performed by the operator and of the well-being of the treatment administered. 43 women declared a feeling of well-being, only 4 did not feel anything and 3 declared "other". In addition, all 49 women said they would return to the treatment, and 1 was dissatisfied (see Figure 2). Following OMT, none of the full-term pregnant women reported adverse events and triggers of labor.

Table II. Main descriptive statistics of pre- and post-OMT means OT salivary concentrations and the change in mean OT salivary concentration following the OMT.

OT salivary concentration (pg/mL) values	Pre-OOMT (A)	Post-OMT (B)	B – A	*p*-value
Mean	89.98	100.60	10.62	**< 0.000005**
Median	84.25	99.13	8.64	-
Standard Deviation	16.39	19.13	13.54	-
Asimettry Index	0.40	0.80	0.52	-
Range	62.11	84.42	60.75	-
Min	59.78	69.98	-15.72	-
Max	121.89	154.40	45.03	-

Table III. Multivariate analysis conducted on the change in mean OT salivary concentration following OMT between different patient clusters.

	B – A	*p*-value
Italian *vs.* foreign	11.21 *vs.* 6.31	0.32
Normalweight *vs.* overweight	10.73 *vs.* 10.75	0.96
Allergic *vs.* non	9.89 *vs.* 10.94	0.80
Nulliparous *vs.* pluriparous	10.25 *vs.* 11.14	0.88
37^{th}-39^{th} *vs.* 40^{th}-41^{st}	11.43 *vs.* 8.74	0.55
ART pregnancy *vs.* spontaneous	14.32 *vs.* 10.21	0.62
Painful contractions *vs.* non	19.76 *vs.* 8.34	**0.06**
Morning OMT *vs.* afternoon	9.38 *vs.* 11.68	0.55
Under 35 *vs.* over 35	13.84 *vs.* 7.41	**0.09**

Discussion

Several studies[5,7,16,17] have reported an increase in the concentration of OT in saliva, blood and urine in response to social, affective contact in chimpanzees and humans. Still, no evidence defines a specific role of massage in pregnant women, in particular, that evaluates to which extent a massage can affect the production of OT.

This is the first study to assess whether OMT can modify endogenous OT production in full-term pregnant women, giving them a feeling of well-being without triggering labor. It demonstrated the importance and effectiveness of OMT regarding the production of OT. In this paper, for the women who reported painful contractions and women aged less than 35 years, there was a greater increase in the mean OT salivary concentration post-OMT. In the first case, it can be assumed that, since the painful contractions could be a sign of the presence in the circulation of endogenous OT, the OMT has contributed to further raising its values. It should be emphasized that the response to OMT also depends on the operator who performs it, on his empathic qualities towards the woman, and on the operator's attention in avoiding generating situations that could undermine the relationship with patients. There is an interesting correlation between these operator requirements and the correlation between the average increase in salivary OT concentration found in the treated sample and the percentage of patients satisfied at the end of OMT (confirmed by the satisfaction questionnaire). The feeling of well-being found in the majority of patients indicates that there was a prevalently central rather than peripheral release of OT, which indicates that there has been a predominantly central, rather than peripheral OT release. Contextual and interindividual factors moderate the effects of OT, as well as peripheral OT levels[18].

The analysis of results reported that the levels of OT produced by patients are significantly higher in those with the lowest starting values, thus demonstrating the effectiveness of the treatment. Importantly, the results collected in our study were obtained using salivary swabs, because human plasma assays necessitate invasive methods. OT measures are often limited by these technical requirements and are confined to clinical settings where blood sampling can be conducted by personnel trained in phlebotomy methods. In addition, plasma OT concentrations obtained by individual blood draws are likely to be contaminated by a stress-induced OT increase in response to the stress of the needle stick. Thus, an indwelling venous catheter and a long habituation period before sampling are necessary to measure true resting levels and to maximize changes elicited solely by experimental manipulation. These difficulties make less invasive measurement of OT in saliva an attractive alternative, and our choice is supported by recent studies[19,20] that show like OT values are reliable when measured in saliva by immunoenzymatic test. A study on the reliability of the results of this type of data collection highlights how salivary and plasma OT levels and "ranges" are similar, albeit moderately higher in saliva[21].

However, the study has several limitations, including a low number of patients and a single center design of the study. Another limiting factor was the measurement of salivary and non-plasma oxytocin levels. A final limiting factor was the absence of a control group and/or randomization with other types of osteopathic manipulative treatment. Nonetheless, this study used a single operator for the OMT that removes the bias of

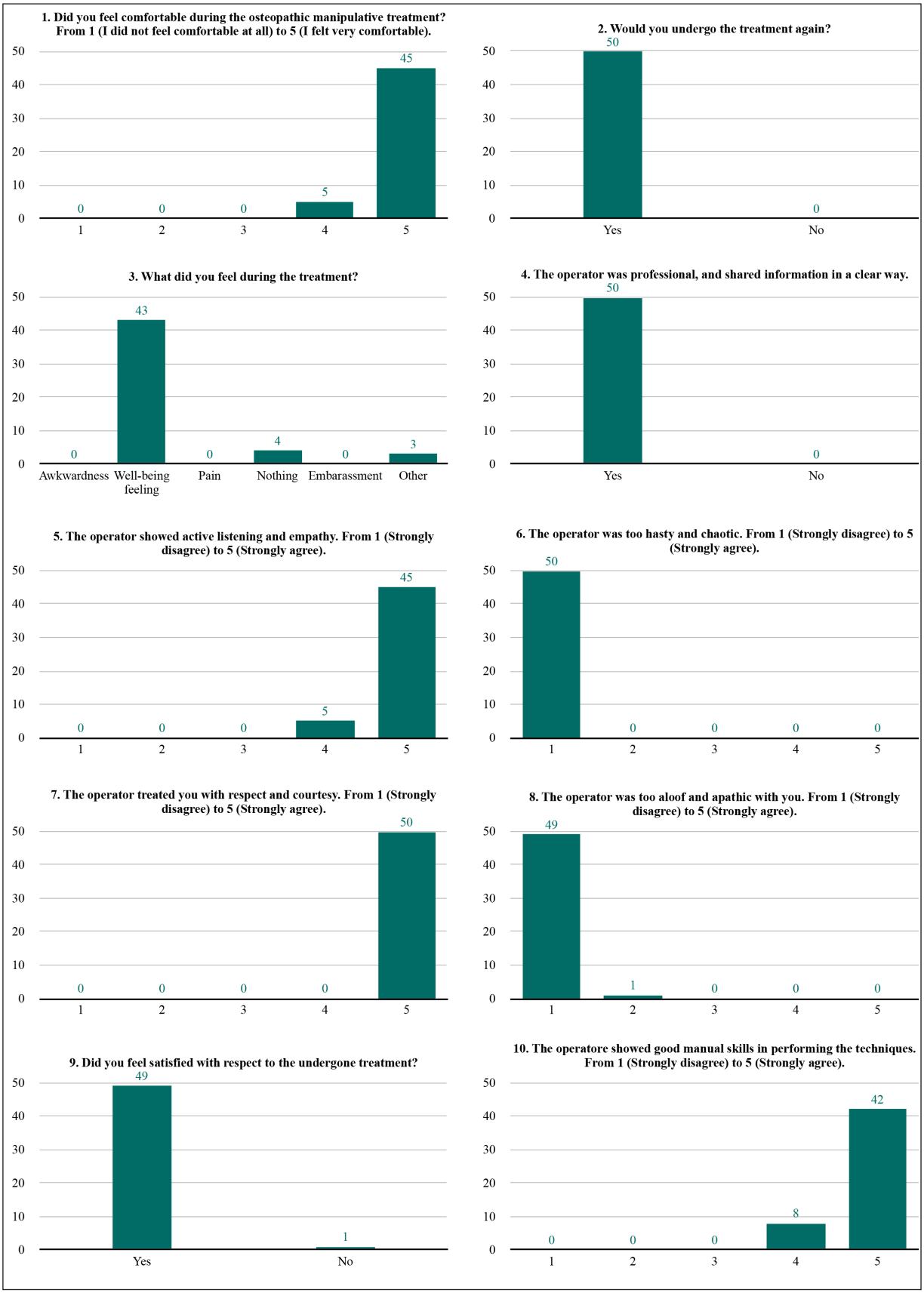

Figure 2. Mapping of patient satisfaction questionnaire response.

different operators, the double evaluation of the swabs analyzed, and performed the average for a single swab result to have a certain result.

Finally, because there are no studies in the literature regarding the role of osteopathy on endogenous incretion of oxytocin in full-term pregnant women, it cannot be said with certainty that the results obtained in this study are uniquely attributable to the performance of the OMT, as they could also be ascribed to the empathetic "touch" of the operator due to the result of the questionnaires administered which showed great satisfaction of women regarding this point[22].

The possibility of introducing OMT to reduce the administration of intravenous SynOT in patients in the latent phase of labor, or the use in patients suffering from dystocia of labor, is more complex and is to be evaluated with subsequent studies. Current obstetric medical literature is characterized by studies focusing on dosage (high/low dose) for administering SynOT for labor augmentation/induction and for preventing and treating postpartum hemorrhage in human birth[23-26]. Furthermore, SynOT does not produce the same beneficial effects as endogenous oxytocin in the brain, as it cannot cross the blood-brain barrier[27].

Conclusions

This longitudinal clinical study demonstrates OMT's effectiveness for the first time, increasing endogenous oxytocin statistically significant in full-term pregnant women. In these women, the increase in endogenous oxytocin did not result in triggering labor. Therefore, women could benefit from the positive effects, and OMT should be considered for further studies as a possible alternative for increasing the endogenous incretion of OT without the side effects of binding to SynOT administration.

Conflict of Interest

The authors declare that they have no conflict of interest to declare.

Ethics Approval

This study was conducted following the regulatory standards of Good Clinical Practice and the Declaration of Helsinki and was approved by the Internal Review Board of Campus Bio-Medico of Rome (Prot. No. 66/2022).

Informed Consent

Informed consent was obtained from all study participants.

Authors' Contribution

A. R., A. S., M. F., F. F., F. P., C. D. L., S. D. A., F. D., M. D. C., G. M., A. B., S. A., R. A., C. T. conceived and designed the experiments, data collection, analyzed the data, and wrote the manuscript. All authors read and approved the final manuscript.

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Data Availability Statement

The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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