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Association between Disgust Sensitivity during Pregnancy and Endogenous Steroids: A Longitudinal Study

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Abstract: The emotion of disgust protects individuals against pathogens, and it has been found that during pregnancy, it is elevated. Physiological mechanisms discussed in relation to these changes include immune markers and progesterone levels. The aim of this study is to assess the association between steroids and disgust in pregnancy. In a sample of 179 pregnant women, we have analyzed blood serum steroid concentrations and measured disgust sensitivity by text-based questionnaires in the first and third trimester. We found positive correlations between disgust sensitivity and the levels of C19 steroids, including testosterone, but also its precursors in the Δ^5 pathway (androstenediol, DHEA, and their sulfates) and the Δ^4 pathway (androstenedione). Furthermore, positive correlations between disgust sensitivity and 5α/β-reduced C19 steroid metabolites were consistently present in both trimesters. In the first trimester, we found a positive association between disgust sensitivity and 17-hydroxypregnanolone as well as some estrogens. In the third trimester, we have observed positive associations between disgust sensitivity and cortisol as well as immunoprotective Δ^5 C19 7α/β-hydroxy-steroids. Our study has thus shown that disgust sensitivity is positively correlated with immunomodulatory steroids, and in the third trimester, steroids may reflect potential maternal anxiety-related symptoms.

Keywords: steroids; disgust; pregnancy; behavioral immune system; testosterone; estrogens; androstenediol; DHEA; 7α/β-hydroxy-androgens; cortisol

1. Introduction

In recent decades, there developed at the intersection of psychology and immunology a growing interest in the behavioral immune system, a network of psychological mechanisms that serve as the first line of defense against potential pathogens from the environment. The term describes adaptive human behavioral reaction to various infectious threats. The affective part of the behavioral immune system consists of the emotion of disgust [1], which is linked to avoidance of potentially harmful stimuli [2].

When it comes to disgust sensitivity, one can observe both interindividual and intraindividual differences. It has been shown that disgust sensitivity changes during ontogenesis [3,4] and differences in disgust sensitivity have also been observed between men and women [2,5]. Moreover, disgust sensitivity can be influenced by numerous factors, including activity of the immune system [6,7], the presence of pathogens in the environment [8], reproductive status [9,10], and psychosocial influences [11,12]. Based on the premise of this variability in disgust sensitivity, the compensatory

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prophylaxis hypothesis has been proposed [13], assuming that the individual level of sensitivity to disgust is modulated depending on the current degree of immunosuppression.

Originally, the hypothesis was developed within the context of variations in the levels of progesterone, a hormone believed to have immunosuppressive effects, and the associated impact on immunosuppression during the menstrual cycle [14]. During the luteal phase, when progesterone levels reach their peak, it is believed that increased disgust sensitivity compensates for the associated increased immunosuppression. In subsequent years, numerous studies have tested the compensatory prophylaxis hypothesis in the context of the menstrual cycle and in relation to changes in the levels of progesterone (and other hormones). The reported findings did not, however, form a consistent pattern.

Some studies that investigated this hypothesis focused on changes in disgust sensitivity during the menstrual cycle, especially on differences between the different phases of the cycle. The first cross-sectional study to investigate this hypothesis, which was also the first study to compare disgust sensitivity in the follicular and luteal phase, found no difference when disgust was measured by the Disgust Scale [13]. Similarly, later cross-sectional studies – one that used video stimuli to elicit disgust [15], another that applied two different disgust scales [16], and two longitudinal studies, one of which again used video stimuli [17] and the other used the Three Domains of Disgust Scale [18] – found no evidence in support of the compensatory prophylaxis hypothesis. The results of a cross-sectional study by Rafiee et al. [19], which observed the relationship between estimated (based on the calculated day of the cycle) levels of progesterone and estradiol, and scores on the Pathogen domain of the Three Domains of Disgust Scale, also found no supporting evidence. On the other hand, the results of two other longitudinal studies do support the compensatory prophylaxis hypothesis: they found that women had higher disgust sensitivity during the luteal phase than during menstruation [20]or during the follicular phase [21]. A cross-sectional study also found elevated disgust sensitivity during the luteal phase in a subsample of women who recently had an infection [22]. In a recent study, women in the luteal phase displayed a more negative attitude and higher sensitivity to disgust-related phrases compared to women tested during the follicular phase or menstruation [23].

Changes in disgust sensitivity during the menstrual cycle have also been observed in direct relation to hormone levels. The currently leading hypothesis claims that higher levels of progesterone are associated with higher disgust sensitivity. The first cross-sectional study found a positive association between the levels of salivary progesterone and disgust sensitivity to visual stimuli [24]. In a longitudinal study, progesterone levels in the serum positively correlated with disgust sensitivity as measured by the Disgust Scale-Revised (DS-R) and the Pathogen domain of the Three Domains of Disgust Scale (TDDS) but only during the mid-luteal phase [20]. While not directly measuring disgust sensitivity, two studies focused on the processing of disgusted facial expressions. One found a positive association between the levels of salivary progesterone and a higher tendency to perceive disgusted faces with averted gaze as more intense, which could signify an increased sensitivity to facial cues signaling a nearby presence of a pathogenic threat [25] . Another study found a negative association between salivary estradiol levels and the overall processing of disgusted faces with direct gaze, which is thought to express direct communication of disgust over violation of moral norms [26]. The authors suggest that progesterone and estradiol could modulate the perception of disgusted faces differently depending on the direction of gaze. There is some evidence for a link between progesterone and disgust sensitivity in animal models as well: a reappraisal of data from a recent study on mice by Kavaliers et al. [27] had shown that an injection of progesterone given to females increased disgust towards infected males (as measured by the frequency of females avoiding the odor of infected males) [28].

On the other hand, multiple studies did not find the expected association, including Timmers et al. [17], who found no association between changes in the levels of salivary progesterone and changes in self-reported disgust, both measured repeatedly in the follicular and luteal phase of the cycle. A longitudinal study that measured not only salivary progesterone but also estradiol, testosterone, and cortisol, found no association between either of the hormones and the TDDS [29]. Another recent longitudinal study likewise measured the levels of salivary progesterone, estradiol, testosterone, and

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cortisol and found no association with disgust sensitivity as measured by the Pathogen domain of the TDDS, neither in within-subject nor in between-subject analyses [18].

Simultaneously with the aforementioned research, Fessler et al. [9] have extended investigation of the compensatory prophylaxis hypothesis to pregnant women. They hypothesized that disgust sensitivity would be elevated during the first trimester of pregnancy: it would be consistent with older theories which assumed that women's immune system is suppressed during this phase of pregnancy. While their assumptions were confirmed in both their cross-sectional study [9] and in another longitudinal study [30], it is essential to understand how our understanding of immunosuppression in early pregnancy had evolved. Recent findings indicate that during early pregnancy, the maternal immune system undergoes complex immunomodulation, with some processes being suppressed and others (such as inflammatory processes) being elevated [31–33]. Despite that – and in accordance with the compensatory prophylaxis hypothesis – increased susceptibility to disgust was recently observed in early pregnancy in women with lower levels of certain cytokines, in women whose immune system is probably insufficiently activated [34], and in those who had lower maternal serum levels of free β-human chorionic gonadotropin (associated with pregnancy-induced immunotolerance) [7]. During the first trimester, a higher disgust sensitivity was also observed in women who reported recent health problems [10] or in association with increased concentrations of pathogens in the environment, such as during the COVID-19 pandemic [7]. Moreover, a recent study by Dlouhá et al. [16] showed a significantly higher disgust sensitivity in women during early pregnancy compared with non-pregnant childless controls, which indicates that higher disgust sensitivity in pregnancy may provide protection during a period that is sensitive to fetal neurodevelopmental disruptions.

Although two studies [9,30] found higher disgust sensitivity during the first trimester than in later pregnancy, Dlouhá et al. [10] have in a recent longitudinal study found increasing disgust sensitivity throughout pregnancy and even after birth. The authors of the study discussed a possible association with increasing progesterone during pregnancy [14] but progesterone levels decrease after childbirth, which is not consistent with the reported further increase in disgust sensitivity during the postpartum period. The increase might be attributed to a more intense need for protection against infections towards the end of pregnancy due to the approaching childbirth and subsequent care for the newborn. Another potential interpretation of these findings revolves around a positive correlation between disgust sensitivity and negative affectivity. Existing research indicates that disgust is linked to affective states such as phobias [35,36], depression [11], or anxiety [37], and that alterations in disgust levels coincide with changes in the symptoms associated with contamination-based obsessive-compulsive disorder [12]. It is known that late pregnancy is associated with increased anxiety symptoms [38] and elevated fear of death has been observed in the third trimester [39] and during labor [40]. It has been shown that these factors, along with anxiety, are related to postpartum anxiety [41].

It is apparent from the above that the relation between hormone levels and changes in disgust sensitivity, especially in women during the menstrual cycle, has been repeatedly discussed and the results are inconsistent. On the other hand, in the context of pregnancy, although it is accompanied by significant hormonal and immunological changes, only one study to date has explored the influence of hormones on disgust sensitivity [7]. To better understand the association between hormonal changes and changes in disgust sensitivity and in order to shed more light on the proximal mechanisms of disgust regulation during pregnancy, we have tested a broad range of steroid hormones in the first and then again in the third trimester of pregnancy. We focused on associations between disgust sensitivity and the levels of hormones with immunomodulatory effects, such as progesterone, testosterone, cortisol, estradiol, or 7 oxygenated (7α -hydroxy, 7β-hydroxy, and 7-oxo) and 16α-hydroxy-derivatives of adrenal androgens dehydroepiandrosterone (DHEA) and 5-androstene-3β,17β-diol [42,43]. We have also carried out an explorative analysis regarding possible associations between disgust and the levels of other endogenous steroids. Previous research that investigated the circulating steroids accompanying various mental disturbances [43] suggests that the relationship between disgust levels, immunity, and psychological status may also be modulated

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by, for instance, estrogen levels, reduced sulfoconjugation of steroids, 7α-, 7β- and 16α-hydroxy-metabolites of C19 Delta(5) steroids, or 5α/β-reduced pregnane steroids.

2. Results

2.1. Descriptive Statistics

The final sample consisted of 179 women aged between 21 and 44 (Mean = 31.5, SD = 4.27), out of whom 109 (60.9%) were primiparous, and 75 (41.9%) were pregnant with a male fetus. Most women were from the ProfiGyn clinic (n = 133), 37 women from the GynFleur clinic and only 9 women were recruited in the Levret clinic. Most women had a university degree of education (78.8%) and were married (54.7%). Eight (5%) women had hypertension and 19 (11.9%) women had gestational diabetes mellitus. Out of the sample, 16 women (8.9%) reported that they smoked regularly before pregnancy and 18 women (10.1%) only occasionally. The levels of steroids quantified in the circulation of pregnant women in the 1st and 3rd trimester are reported in Table 1.

Table 1. Characteristics of pregnant women and serum steroid levels.

Steroids (nM)	Trimester 1 median(quartiles)	Trimester 3 median(quartiles)	Trimester 3 – Trimester 1 median(quartiles)	p-value
C21 Δ⁵ Steroids				
Pregnenolone	4.28 (2.86, 6.22)	4.84 (3.46, 7.32)	0.563 (-0.812, 2.99)	<0.001
Pregnenolone sulfate	180 (124, 259)	212 (161, 295)	32.6 (-14.1, 84.4)	<0.001
20α-Dihydropregnenolone	4.78 (3.55, 6.09)	3.29 (2.66, 4.32)	-1.23 (-2.44, -0.327)	<0.001
20α-Dihydropregnenolone sulfate	954 (639, 1390)	849 (620, 1100)	-125 (-378, 92.5)	<0.001
17-Hydroxypregnenolone sulfate	4.6 (3.07, 7.9)	2.69 (2.01, 3.98)	-2.04 (-4.86, -0.636)	<0.001
17-Hydroxypregnenolone	7.29 (4.63, 12)	7.39 (5.05, 11.5)	0.144 (-3.54, 3.08)	0.704
16α-Hydroxypregnenolone	0.462 (0.298, 0.68)	0.806 (0.589, 1.13)	0.344 (0.0694, 0.593)	<0.001
C19 Δ⁵ Steroids				
Dehydroepiandrosterone (DHEA)	9.25 (6.01, 12.9)	4.63 (3.29, 6.87)	-4.12 (-6.63, -1.66)	<0.001
DHEA sulfate	2900 (1800, 4360)	1150 (666, 1810)	-1670 (-2560, -910)	<0.001
7α-Hydroxy-DHEA	0.909 (0.539, 1.43)	0.344 (0.228, 0.526)	-0.533 (-0.973, -0.233)	<0.001
7-oxo-DHEA	0.503 (0.306, 0.842)	0.519 (0.41, 0.71)	0.0141 (-0.307, 0.235)	0.647
7β-Hydroxy-DHEA	0.598 (0.342, 0.885)	0.285 (0.175, 0.382)	-0.334 (-0.565, -0.124)	<0.001
Androstenediol	2.72 (1.85, 4.1)	1.13 (0.741, 2.15)	-1.24 (-2.16, -0.51)	<0.001
Androstenediol sulfate	322 (194, 512)	174 (112, 261)	-130 (-307, -25.3)	<0.001
5-Androstene-3β,7α,17β-triol	0.459 (0.281, 0.729)	0.123 (0.084, 0.233)	-0.305 (-0.571, -0.165)	<0.001
5-Androstene-3β,7β,17β-triol	0.509 (0.319, 0.873)	0.231 (0.144, 0.319)	-0.312 (-0.572, -0.134)	<0.001
5-Androstene-3β,16α,17β-triol	0.206 (0.131, 0.319)	0.496 (0.337, 0.788)	0.266 (0.107, 0.536)	<0.001
5-Androstene-3β,16α,17β-triol sulfate	32.6 (18.9, 61.6)	78.2 (46.9, 139)	34 (10.5, 78.2)	<0.001
C21 Δ⁴ Steroids				
Progesterone	104 (76.5, 135)	382 (281, 489)	277 (183, 390)	<0.001
20α-Dihydroprogesterone	22.5 (17.2, 29.2)	49.2 (36.3, 67.7)	26.3 (15.6, 43)	<0.001
17-Hydroxyprogesterone	11 (8.47, 14.1)	17.4 (13.8, 21.3)	5.7 (1.69, 10.9)	<0.001
16α-Hydroxyprogesterone	2.14 (1.57, 2.79)	10.2 (7.62, 14.3)	8.07 (5.31, 12.2)	<0.001
17α,20α-Dihydroxy-4-pregnene-3-one	3.08 (2.18, 4.12)	4.44 (3.34, 5.85)	1.25 (0.332, 2.54)	<0.001
C19 Δ⁴ Steroids				
Androstenedione	7.22 (5.44, 9.79)	6.62 (4.8, 9.26)	-0.966 (-2.54, 0.667)	<0.001
Testosterone	2.9 (2.1, 3.9)	2.54 (1.69, 4.17)	-0.245 (-0.805, 0.784)	0.308
Conjugated testosterone	1.08 (0.407, 2.39)	4.2 (2.12, 7.29)	2.1 (0.335, 5.67)	<0.001
5α-Dihydrotestosterone	0.903 (0.596, 1.45)	0.443 (0.27, 0.682)	-0.446 (-0.761, -0.167)	<0.001
Conjugated 5α-dihydrotestosterone	1.42 (0.854, 2.09)	1 (0.677, 1.58)	-0.288 (-1.09, 0.364)	<0.001
Estrogens				
Estrone	3.57 (1.99, 6.85)	17 (10.4, 31.4)	13.4 (5.26, 29.9)	<0.001
Estrone sulfate	5.67 (3, 11.7)	27.3 (16.8, 47.6)	21.7 (9.41, 38.2)	<0.001
Estradiol	3.31 (1.55, 5.77)	31 (18.4, 48.5)	27 (16.2, 44.4)	<0.001
Estradiol sulfate	3.98 (2.18, 6.83)	19.8 (10.3, 35.2)	15.8 (4.91, 29.6)	<0.001
Estriol	1.89 (0.726, 4.44)	74.8 (40.8, 119)	74.3 (40.9, 116)	<0.001
C21 5α/β-reduced Steroids				
5α-Dihydroprogesterone	10.4 (7.09, 13.7)	65.1 (42.8, 92.5)	54.3 (30.7, 80.6)	<0.001
Allopregnanolone	6.71 (5.05, 9.09)	31.1 (21.1, 42.7)	23.8 (14.8, 34)	<0.001
Allopregnanolone sulfate	128 (82.7, 227)	1220 (739, 1870)	1080 (534, 1690)	<0.001
Isopregnanolone	2.05 (1.38, 2.86)	7.96 (4.44, 11.9)	5.28 (2.73, 9.51)	<0.001

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Isopregnanolone sulfate	92.3 (59, 143)	605 (344, 958)	477 (263, 837)	<0.001
5β-Dihydroprogesterone	0.123 (0.043, 0.235)	2.02 (1.1, 3.26)	1.89 (0.978, 3.01)	<0.001
Pregnanolone	1.91 (1.06, 3.06)	22.8 (14.5, 29.9)	20 (13.5, 27.1)	<0.001
Conjugated pregnanolone	76 (54, 131)	696 (480, 950)	549 (361, 806)	<0.001
Epipregnanolone	0.267 (0.137, 0.538)	1.36 (0.823, 1.97)	0.935 (0.596, 1.53)	<0.001
Conjugated epipregnanolone	23.7 (14.7, 37.3)	154 (95.4, 255)	117 (67.8, 207)	<0.001
17-Hydroxyallopregnanolone	0.11 (0.04, 0.197)	0.279 (0.124, 0.511)	0.149 (0.019, 0.314)	<0.001
17-Hydroxyallopregnanolone sulfate	6.6 (3.82, 11)	11.1 (6.83, 19.5)	3.48 (0.727, 9.93)	<0.001
17-Hydroxypregnanolone	0.195 (0.103, 0.36)	0.882 (0.595, 1.19)	0.623 (0.388, 0.899)	<0.001
Conjugated 17α-hydroxypregnanolone	24.1 (16.8, 39.6)	46.4 (31, 70.6)	19.4 (8.95, 35.1)	<0.001
5α,20α-Tetrahydroprogesterone	6.04 (4.42, 8.2)	21.7 (15.1, 31.1)	15.3 (9.37, 22.2)	<0.001
Conjugated 5α,20α-tetrahydroprogesterone	5.35 (3.3, 8.59)	15.5 (10.7, 25.2)	10.1 (4.74, 18.5)	<0.001
5α-Pregnane-3α,20α-diol	7.23 (4.83, 9.68)	23.1 (16, 32.4)	14.2 (8.53, 23.9)	<0.001
Conjugated 5α-pregnane-3α,20α-diol	1650 (1100, 2580)	6550 (4220, 9420)	4440 (2660, 7260)	<0.001
5α-Pregnane-3β,20α-diol	2.24 (1.57, 3.27)	6.59 (4.35, 10.5)	3.65 (2.33, 7.26)	<0.001
Conjugated 5α-pregnane-3β,20α-diol	3720 (2150, 6210)	10900 (7600, 17000)	6550 (3540, 11500)	<0.001
5β,20α-Tetrahydroprogesterone	0.096 (0.062, 0.171)	1.96 (1.4, 2.66)	1.8 (1.28, 2.47)	<0.001
Conjugated 5β,20α-tetrahydroprogesterone	2.65 (1.68, 4.06)	7.48 (5.02, 9.64)	4.22 (2.05, 6.78)	<0.001
5β-Pregnane-3α,20α-diol	0.829 (0.584, 1.21)	6.61 (5.06, 8.53)	5.53 (4.19, 7.16)	<0.001
Conjugated 5β-pregnane-3α,20α-diol	297 (229, 427)	1070 (794, 1460)	750 (498, 998)	<0.001
5β-Pregnane-3β,20α-diol	0.18 (0.113, 0.313)	0.593 (0.396, 0.927)	0.393 (0.209, 0.665)	<0.001
Conjugated 5β-pregnane-3β,20α-diol	189 (127, 286)	644 (461, 920)	430 (253, 624)	<0.001
5α-Pregnane-3α,17α,20α-triol	0.276 (0.146, 0.474)	0.179 (0.104, 0.321)	-0.082 (-0.199, 0.006)	<0.001
Conjugated 5α-pregnane-3α,17α,20α-triol	28.5 (10.5, 58)	35.5 (11.8, 87.5)	3.2 (-5.57, 23.6)	0.001
5β-Pregnane-3α,17α,20α-triol	2.8 (1.88, 3.94)	4.79 (3.16, 6.17)	1.52 (0.35, 2.82)	<0.001
Conjugated 5β-pregnane-3α,17α,20α-triol	116 (82.2, 216)	162 (117, 269)	34.8 (-21.4, 102)	<0.001
5α-Androstane-3,17-dione	0.521 (0.34, 0.744)	0.425 (0.297, 0.625)	-0.0559 (-0.197, 0.0546)	<0.001
C19 5α/β-reduced Steroids				
Androsterone	0.897 (0.705, 1.21)	0.568 (0.427, 0.815)	-0.336 (-0.549, -0.12)	<0.001
Androsterone sulfate	1290 (718, 2100)	594 (333, 986)	-540 (-1250, -258)	<0.001
Epiandrosterone	0.333 (0.205, 0.497)	0.158 (0.102, 0.251)	-0.16 (-0.288, -0.062)	<0.001
Epiandrosterone sulfate	348 (214, 496)	129 (76.8, 187)	-199 (-312, -116)	<0.001
Etiocholanolone	0.265 (0.164, 0.421)	0.233 (0.148, 0.396)	-0.025 (-0.103, 0.062)	0.103
Etiocholanolone sulfate	53.5 (34.2, 84.6)	36.6 (20, 56.2)	-15.6 (-36.2, -4.48)	<0.001
Epietiocholanolone sulfate	17 (10.4, 35.5)	9.45 (6.06, 17.8)	-7.24 (-18.5, -2.09)	<0.001
5α-Androstane-3α,17β-diol	0.174 (0.118, 0.245)	0.079 (0.061, 0.11)	-0.09 (-0.145, -0.05)	<0.001
Conjugated 5α-androstane-3α,17β-diol	19.7 (14.2, 28.7)	13.2 (8.46, 18)	-7.12 (-14.7, -1.3)	<0.001
5α-Androstane-3β,17β-diol	0.064 (0.024, 0.141)	0.032 (0.01, 0.075	-0.022 (-0.081, -0.002)	<0.001
Conjugated 5α-androstane-3β,17β-diol	26.6 (16.1, 56.5)	12.5 (7.12, 23.6)	-14.8 (-35.1, -4.69)	<0.001
5β-Androstane-3α,17β-diol	0.011 (0.006, 0.018)	0.007 (0.003, 0.013)	-0.004 (-0.009, 0.002)	<0.001
Conjugated 5β-androstane-3α,17β-diol	3.98 (2.5, 6.59)	2.85 (1.78, 4.9)	-0.916 (-2.09, 0.272)	<0.001
Corticoids and 11β-hydroxy-androstanes				
Cortisol	389 (308, 473)	632 (537, 824)	231 (148, 386)	<0.001
Cortisone	106 (77.1, 148)	170 (128, 252)	52.8 (22.8, 111)	<0.001
Corticosterone	12.5 (7.38, 18.6)	19.5 (14.6, 27.2)	6.96 (-0.918, 14.7)	<0.001
11-Deoxycortisol	0.74 (0.168, 1.87)	3.49 (1.06, 7.02)	2.57 (0.423, 5.22)	<0.001
21-Deoxycortisol	0.081 (0.029, 0.243)	0.117 (0.0656, 0.259)	0.029 (-0.025, 0.098)	<0.001
3α,5α-Tetrahydrocorticosterone	0.039 (0.0173, 0.087)	0.028 (0.010, 0.056)	-0.009 (-0.043, 0.003)	<0.001
3α,5β-Tetrahydrocorticosterone	0.124 (0.0409, 0.328)	0.06 (0.022, 0.127)	-0.052 (-0.228, 0.0112)	<0.001
11β-Hydroxyandrostenedione	48.3 (29.9, 74.9)	60 (39.5, 102)	8.58 (-1.77, 30)	<0.001
11β-Hydroxyandrosterone	1.2 (0.702, 2.14)	0.316 (0.188, 0.528)	-0.897 (-1.73, -0.432)	<0.001
11β-Hydroxyandrosterone sulfate	12.2 (8.5, 18.3)	7.45 (4.59, 11.6)	-4.56 (-9.27, -0.811)	<0.001
11β-Hydroxyepiandrosterone	0.048 (0.023, 0.101)	0.012 (0.005, 0.025)	-0.031 (-0.074, -0.009)	<0.001
11β-Hydroxyepiandrosterone sulfate	0.783 (0.413, 1.25)	1.81 (0.938, 3.18)	0.974 (0.203, 1.94)	<0.001
11β-Hydroxyetiocholanolone	0.927 (0.576, 1.39)	0.422 (0.241, 0.673)	-0.44 (-0.799, -0.213)	<0.001
11β-Hydroxyetiocholanolone sulfate	3.01 (1.82, 4.85)	1.77 (1.05, 2.93)	-1.06 (-2.17, -0.174)	<0.001

Note: p-values show significant differences between trimesters.

Disgust sensitivity was assessed by the Disgust Scale-Revised (DS-R) [44] and by the Pathogen domain of the Three Domains of Disgust Scale (TDDS) [45] (for more details, see the Material and Methods section). The mean DS-R/TDDS scores and internal consistencies are shown in Table 2.

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Table 2. Descriptive statistics for the DS-R (Core, Animal reminder, Contamination disgust subscale scores and the overall DS-R score) and the Pathogen domain of the TDDS in the first (T1) and third (T3) trimester.

Questionnaire	n	Mean	Median (quartiles)	SD	Min.	Max.	Cronbach's alpha
Overall DS-R T1	169	56.2	55 (45, 66)	14.3	21	93	0.843
Overall DS-R T3	176	55.1	56 (44.8, 66)	15.3	15	91	0.874
Core T1	169	29.4	30 (25, 35)	6.96	11	48	0.676
Core T3	176	28.4	28 (23, 34)	7.56	8	47	0.763
Contamination T1	169	8.53	8 (6, 11)	3.62	2	19	0.562
Contamination T3	176	8.53	9 (6,11)	3.70	0	19	0.622
Animal reminder T1	169	18.3	18 (14, 23)	6.37	3	32	0.760
Animal reminder T3	176	18.2	19 (13, 23)	6.66	0	32	0.792
Pathogen TDDS T1	174	23.3	24 (17, 28)	7.35	4	40	0.734
Pathogen TDDS T3	176	23.4	23 (18, 29)	7.70	7	41	0.798

Note: The table shows the total number of participants that filled out each questionnaire at each time point.

2.2. Association between Disgust Sensitivity and Steroid Levels in the 1st Trimester

We assessed the association between disgust sensitivity and steroid levels in the 1st trimester of pregnancy. In the OPLS model for the overall DS-R score, disgust was significantly predicted by a broad spectrum of steroids, such that higher levels of androstenediol, 17-hydroxypregnanolone, 5β-pregnane-3α,17α,20α-triol, 5α-androstane-3,17-dione, androsterone, androsterone sulfate, epiandrosterone, epiandrosterone sulfate, 5α-androstane-3α,17β-diol, 5α-androstane-3β,17β-diol, 5β-androstane-3α,17β-diol, 3α,5β-tetrahydrocorticosterone, and lower levels of estrone predicted higher overall DS-R score. This model explained 9.8% (6.8% after cross-validation) of the overall DS-R score variability (Table 3).

The model for the Core disgust subscale revealed that higher levels of androstenediol, testosterone, 5α-dihydrotestosterone, 5α-androstane-3,17-dione, androsterone, androsterone sulfate, epiandrosterone sulfate, 5α-androstane-3α,17β-diol, conjugated 5α-androstane-3β,17β-diol, and lower levels of conjugated pregnanolone predicted higher scores of Core disgust. This model explained 11.4% (9.7% after cross-validation) of the Core disgust score variability (Table 3).

The model for the Contamination disgust subscale revealed that higher subscale scores were predicted by higher levels of estrone, androsterone sulfate, epiandrosterone sulfate, etiocholanolone sulfate, epitetiocholanolone sulfate, and conjugated 5α-androstane-3β,17β-diol. This model explained 7.6% (4.8 % after cross-validation) of the Contamination disgust score variability (Table 3).

The model for the Animal reminder disgust subscale showed that the scores of this subscale were positively associated with the following steroids: testosterone, estrone, estradiol, 5β,20α-tetrahydroprogesterone, conjugated 5β-androstane-3α,17β-diol, and 5α-androstane-3,17-dione. This model explained 9.2% (7.4% after cross-validation) of the Animal reminder disgust score variability (Table 3).

In the OPLS model for the Pathogen disgust score of the TDDS, higher disgust was significantly predicted by higher levels of 5α-dihydrotestosterone, estradiol, 5β-pregnane-3α,17α,20α-triol, conjugated 5α-androstane-3β,17β-diol, 5α-androstane-3,17-dione, and lower levels of 5β,20α-tetrahydroprogesterone. This model explained 16.6% (13.5 % after cross-validation) of the Pathogen disgust score variability (Table 4).

Regarding the covariates, no covariates (maternal age, maternal pre-pregnancy BMI, pregnancy length, maternal weight gain, parity, fetal sex, maternal diabetes and hypertension, maternal pre-pregnancy smoking) contributed to the explanation of the variability of the disgust scores measured by both, the DS-R and the Pathogen domain of the TDDS questionnaires.

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Table 3. Associations between disgust sensitivity measured by the DS-R and predictors evaluated by an OPLS model and multiple regression in the first trimester of pregnancy.

Variable	OPLS (predictive component)						Multiple regression		
	Variable importance	t-statistics		Component loading	t-statistics	R^a		Regression coefficient	t-statistics	
DS-R, Overall score										
Androstenediol	0.988	2.91	*	0.289	8.96	0.559	**	0.045	3.32	**
Estrone	0.939	3.24	**	-0.062	-2.43	-0.114	*	-0.042	-2.15	*
17-Hydroxypregnanolone	1.245	2.88	*	0.225	3.18	0.434	**	0.056	2.72	*
5β-Pregnane-3α,17α,20α-triol	0.789	2.04	*	0.228	4.58	0.441	**	0.036	2.60	*
5α-Androstane-3,17-dione	1.025	4.26	**	0.361	11.28	0.697	**	0.046	3.35	**
Androsterone	1.096	3.30	**	0.388	9.55	0.750	**	0.049	3.13	**
Androsterone sulfate	0.981	3.68	**	0.310	11.08	0.595	**	0.044	2.21	*
Epiandrosterone	1.010	2.81	*	0.389	7.71	0.752	**	0.046	1.95	*
Epiandrosterone sulfate	1.092	5.81	**	0.332	7.09	0.640	**	0.049	3.21	**
5α-Androstane-3α,17β-diol	0.822	2.76	*	0.354	8.29	0.686	**	0.037	4.73	**
5α-Androstane-3β,17β-diol	1.007	2.34	*	0.308	10.12	0.595	**	0.045	2.08	*
5β-Androstane-3α,17β-diol	1.082	2.35	*	0.194	4.04	0.369	**	0.049	2.09	*
3α,5β-Tetrahydrocorticosterone	0.825	2.57	*	0.165	3.05	0.320	**	0.037	1.66	
DS-R, Overall score, trimester 1				1.000	2.91	0.313	*			
Explained variability	9.8% (6.8% after cross-validation)									
DS-R, Core disgust										
Androstenediol	0.998	2.34	*	0.305	9.63	0.610	**	0.051	2.14	*
Testosterone	1.199	4.01	**	0.336	6.80	0.672	**	0.061	3.96	**
5α-Dihydrotestosterone	0.974	3.16	**	0.389	16.63	0.780	**	0.050	2.78	*
Estradiol sulfate	0.709	2.36	*	-0.017	-0.41	-0.028		-0.036	-2.56	*
Conjugated pregnanolone	0.598	1.94	*	-0.166	-3.24	-0.327	**	-0.031	-2.06	*
5α-Androstane-3,17-dione	1.110	3.52	**	0.359	5.49	0.719	**	0.057	3.46	**
Androsterone	0.923	8.32	**	0.394	13.88	0.788	**	0.047	7.34	**
Androsterone sulfate	0.988	3.28	**	0.315	5.40	0.632	**	0.051	3.18	**
Epiandrosterone sulfate	0.944	3.92	**	0.334	8.53	0.670	**	0.048	3.79	**
5α-Androstane-3α,17β-diol	1.139	4.40	**	0.409	16.91	0.820	**	0.058	4.31	**
Conjugated 5α-androstane-3β,17β-diol	1.230	6.41	**	0.216	6.00	0.423	**	0.063	6.28	**
DS-R, Core disgust, trimester 1				1.000	7.40	0.338	**			
Explained variability	11.4% (9.7% after cross-validation)									
DS-R, Contamination disgust										
DHEA sulfate	0.474	1.60		0.383	8.32	0.651	**	0.026	1.33	
Conjugated testosterone	0.860	2.73	*	-0.083	-1.21	-0.138		-0.047	-2.13	*
Estrone	0.844	6.58	**	0.216	5.37	0.363	**	0.046	3.91	**
17-Hydroxypregnanolone	0.896	2.29	*	0.090	0.84	0.151		0.049	2.89	*
Androsterone sulfate	1.273	2.75	*	0.468	8.75	0.796	**	0.069	2.42	*
Epiandrosterone sulfate	1.280	3.40	**	0.496	9.37	0.842	**	0.069	2.71	*
Etiocholanolone sulfate	0.826	2.34	*	0.386	12.44	0.659	**	0.045	2.09	*
Epitetiocholanolone sulfate	1.260	2.95	*	0.446	11.03	0.743	**	0.068	2.73	*

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Conjugated 5α-androstane-3β,17β-diol	0.996	2.39	*	0.375	5.34	0.641	**	0.054	1.81		
DS-R, Contamination disgust, trimester 1				1.000	2.14	0.276	*				
Explained variability	7.6% (4.8% after cross-validation)										
DS-R, Animal reminder disgust											
Testoterone	0.752	2.24	*	0.328	2.90	0.505	*	0.061	1.89		
Estrone	1.072	4.66	**	0.450	10.64	0.723	**	0.087	5.98	**	
Estradiol	1.243	6.54	**	0.444	11.46	0.676	**	0.100	4.63	**	
5β,20α-Tetrahydroprogesterone	0.833	2.02	*	0.376	3.65	0.576	**	0.067	2.14	*	
Conjugated 5β-androstane-3α,17β-diol	0.940	3.08	**	0.393	6.55	0.605	**	0.076	2.89	*	
5α-Androstane-3,17-dione	1.078	3.89	**	0.449	8.42	0.690	**	0.087	3.03	**	
DS-R, Animal reminder disgust, trimester 1				1.000	2.57	0.303	*				
Explained variability	9.2% (7.4% after cross-validation)										

[a]R…Component loadings expressed as correlation coefficients with predictive component, *p<0.05, **p<0.01.

Table 4. Associations between Pathogen disgust measured by the TDDS and predictors evaluated by an OPLS model and multiple regression in the first trimester of pregnancy.

	OPLS (predictive component)							**Multiple regression**		
Variable	**Variable importance**	**t-statistics**		**Component loading**	**t-statistics**	**R[a]**		**Regression coefficient**	**t-statistics**	
Testosterone	0.521	1.94	*	0.137	1.27	0.195		0.056	1.71	
5α-Dihydrotestosterone	0.896	3.30	**	0.331	6.40	0.468	**	0.097	2.45	*
Estradiol	0.766	2.72	*	0.168	2.53	0.231	*	0.083	2.66	*
5β,20α-Tetrahydroprogesterone	1.127	3.50	**	-0.326	-2.76	-0.462	*	-0.122	-3.41	**
5β-Pregnane-3α,17α,20α-triol	0.792	2.11	*	0.494	9.74	0.698	**	0.086	1.98	*
Conjugated 5α-androstane-3β,17β-diol	1.565	5.93	**	0.567	8.51	0.800	**	0.169	6.74	**
5α-Androstane-3,17-dione	0.996	6.58	**	0.504	10.66	0.712	**	0.108	4.69	**
TDDS, pathogen disgust in trimester 1				1.000	4.38	0.407	**			
Explained variability	16.6% (13.5% after cross-validation)									

[a]R…Component loadings expressed as correlation coefficients with predictive component, *p<0.05, **p<0.01.

2.3. Association between Disgust Sensitivity and Steroid Levels in the 3rd Trimester

We also assessed the association between disgust sensitivity and steroid levels in the 3rd trimester of pregnancy. In the OPLS model for the overall DS-R score, disgust was significantly predicted by higher levels of 7α-hydroxy-DHEA, androstenediol, 5β-androstane-3α,17β-diol, and cortisol. This model explained 11.7% (8.8% after cross-validation) of the overall DS-R score variability (Table 5).

The model for the Core disgust subscale revealed that higher levels of pregnenolone, 17-hydroxypregnenolone, dehydroepiandrosterone, 7α-hydroxy-DHEA, 7β-hydroxy-DHEA, androstenediol, 5α-androstane-3α,7α,17β-triol, 5α-dihydrotestosterone, epiandrosterone, 5α-androstane-3β,17β-diol predicted higher scores of Core disgust. This model explained 11.7% (8.8% after cross-validation) of the Core disgust score variability (Table 5).

The model for the Contamination disgust subscale revealed that higher subscale scores were predicted by higher levels of pregnenolone, conjugated pregnanolone, conjugated epipregnanolone, conjugated 5α,20α-tetrahydroprogesterone, conjugated 5α-pregnane-3α,20α-diol, conjugated 5β,20α-tetrahydroprogesterone, conjugated 5β-pregnane-3β,20α-diol, and lower levels of testosterone and estradiol sulfate. This model explained 32.5% (23.1% after cross-validation) of the Contamination disgust score variability (Table 5).

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The model for the Animal reminder disgust subscale showed that the scores of this subscale were positively associated with the following steroids: epiandrosterone, 5α-androstane-3β,17β-diol, 5β-androstane-3α,17β-diol, conjugated 5β-androstane-3α,17β-diol. This model explained 10% (7.8% after cross-validation) of the Animal reminder disgust score variability (Table 5).

In the OPLS model for the Pathogen disgust score of the TDDS, higher disgust was significantly predicted by higher levels of DHEA, DHEA sulfate, androstenediol, androsterone sulfate, epiandrosterone, epiandrosterone sulfate, 5β-androstane-3α,17β-diol. This model explained 13% (11.1% after cross-validation) of the Pathogen disgust score variability (Table 6).

As for the covariates, only maternal age and parity contributed to the explanation of the variability of the Contamination disgust subscale score, such that older women, as well as multiparous women, reported higher levels of Contamination disgust.

Table 5. Associations between disgust sensitivity measured by the DS-R and predictors evaluated by an OPLS model and multiple regression in the third trimester of pregnancy.

	OPLS (predictive component)						Multiple regression		
Variable	**Variable importance**	**t-statistics**		**Component loading**	**t-statistics**	**R[a]**		**Regression coefficient**	**t-statistics**	
DS-R, Overall score										
7α-Hydroxy-DHEA	0.757	1.96	*	0.390	8.96	0.626	**	0.091	2.18	*
Androstenediol	0.856	3.01	**	0.356	6.13	0.572	**	0.152	2.69	*
5β-Androstane-3α,17β-diol	1.213	8.34	**	0.401	12.35	0.641	**	0.164	2.37	*
Cortisol	0.961	4.38	**	0.364	7.18	0.584	**	0.137	3.89	**
DS-R, Overall score, trimester 3				1.000	4.30	0.415	**			
Explained variability	11.7% (8.8% after cross-validation)									
DS-R, Core disgust										
Pregnenolone	0.785	2.65	*	0.202	4.88	0.419	**	0.041	1.85	
17-Hydroxypregnenolone	1.361	4.82	**	0.377	10.59	0.782	**	0.071	5.51	**
Dehydroepiandrosterone	0.924	3.14	**	0.386	9.49	0.801	**	0.048	2.66	*
7α-Hydroxy-DHEA	1.153	5.17	**	0.407	16.53	0.844	**	0.060	3.29	**
7β-Hydroxy-DHEA	1.030	4.15	**	0.374	10.36	0.775	**	0.054	2.56	*
Androstenediol	1.125	5.00	**	0.275	8.36	0.569	**	0.059	3.68	**
5-Androstene-3 β,7α,17β-triol	0.984	2.89	*	0.336	11.14	0.696	**	0.051	1.97	*
5α-Dihydrotestosterone	0.566	2.21	*	0.210	6.00	0.434	**	0.029	2.20	*
Epiandrosterone	1.053	3.40	**	0.345	6.85	0.716	**	0.055	6.77	**
5α-Androstane-3β,17β-diol	0.792	2.45	*	0.200	5.18	0.415	**	0.041	1.76	
DS-R, Core disgust, trimester 3				1.000	3.01	0.342	**			
Explained variability	11.7% (8.8% after cross-validation)									
DS-R, Contamination disgust										
Pregnenolone	0.690	3.19	**	0.158	2.33	0.222	*	0.105	2.24	*
Testosterone	1.048	4.14	**	-0.376	-5.44	-0.546	**	-0.151	-4.06	**
Estradiol sulfate	0.950	2.59	*	-0.269	-2.70	-0.393	*	-0.158	-2.06	*
Estriol sulfate	0.636	2.53	*	-0.167	-1.85	-0.255		-0.134	-2.69	*
Conjugated pregnanolone	1.032	5.42	**	0.318	6.55	0.445	**	0.035	0.90	
Conjugated epipregnanolone	1.026	2.79	*	0.310	2.92	0.431	*	0.052	1.08	
Conjugated 5α,20α-tetrahydroprogesterone	1.140	5.96	**	0.301	4.73	0.410	**	0.095	3.07	**

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Conjugated 5α-pregnane-3α,20α-diol	1.131	3.01	**	0.270	3.00	0.368	**	0.103	1.32	
Conjugated 5β,20α-tetrahydroprogesterone	0.978	2.60	*	0.265	2.62	0.362	*	0.080	0.95	
Conjugated 5β-pregnane-3β,20α-diol	0.877	4.29	**	0.282	3.94	0.386	**	0.025	0.73	
Maternal age	1.357	3.74	**	0.390	3.65	0.569	**	0.237	3.60	**
Multipara (1:yes/0:no)	0.925	3.14	**	0.292	2.89	0.428	*	0.185	5.37	**
DS-R, Contamination disgust, trimester 3				1.000	13.56	0.570	**			
Explained variability				32.5% (23.1% after cross-validation)						
DS-R, Animal reminder disgust										
Epiandrosterone	1.080	4.83	**	0.532	7.50	0.676	**	0.135	4.59	**
5α-Androstane-3β,17β-diol	0.894	2.93	*	0.505	6.19	0.641	**	0.112	2.34	*
5β-Androstane-3α,17β-diol	0.951	4.60	**	0.477	7.10	0.605	**	0.119	3.54	**
Conjugated 5β-androstane-3α,17β-diol	1.063	2.98	*	0.489	3.08	0.613	**	0.133	2.50	*
DS-R, Animal reminder disgust, trimester 3				1.000	2.09	0.317	*			
Explained variability				10% (7.8% after cross-validation)						

[a]R…Component loadings expressed as correlation coefficients with predictive component, *p<0.05, **p<0.01.

Table 6. Associations between Pathogen disgust measured by the TDDS and predictors evaluated by an OPLS model and multiple regression in the third trimester of pregnancy.

	OPLS (predictive component)							Multiple regression		
Variable	**Variable importance**	**t-statistics**		**Component loading**	**t-statistics**	**R[a]**		**Regression coefficient**	**t-statistics**	
DHEA	0.737	3.04	**	0.368	5.37	0.667	**	0.056	2.72	*
DHEA sulfate	0.907	3.42	**	0.431	14.40	0.783	**	0.068	3.21	**
Androstenediol	0.533	1.93	*	0.283	4.15	0.513	**	0.040	2.46	*
Androsterone sulfate	1.116	6.79	**	0.428	12.16	0.778	**	0.084	5.63	**
Epiandrosterone	1.383	5.35	**	0.426	10.60	0.773	**	0.104	5.53	**
Epiandrosterone sulfate	1.129	5.90	**	0.465	17.83	0.845	**	0.085	3.84	**
5β-Androstane-3α,17β-diol	0.957	2.90	*	0.250	2.87	0.453	*	0.072	2.17	*
TDDS, Pathogen disgust, trimester 3				1.000	6.00	0.361	**			
Explained variability				13% (11.1% after cross-validation)						

[a]R…Component loadings expressed as correlation coefficients with predictive component, *p<0.05, **p<0.01.

2.4. The Effect of Changes in Steroid Levels during Pregnancy on Changes in Disgust Sensitivity

Finally, we assessed the association between delta scores of disgust (Δdisgust) sensitivity and delta steroids (Δsteroids) calculated as the level measured in the 3rd trimester minus the level measured in the 1st trimester. In the input models, the Δdisgust sensitivity was represented by vector Y. Matrix X was constituted by Δsteroids, steroid levels and relevant disgust sensitivity measured in the first trimester of pregnancy (representing the baseline), and related variables of maternal age, maternal BMI before pregnancy and ΔBMI, pregnancy length (both in the first trimester and delta), maternal weight gain (both in the first trimester and delta), parity, fetal sex, maternal diabetes and hypertension, and maternal pre-pregnancy smoking.

In the OPLS model for Δoverall DS-R score, higher Δdisgust was significantly predicted by higher levels of ΔDHEA sulfate, Δandrostenediol, Δ5α-dihydrotestosterone, Δandrosterone, Δ5α-androstane-3β,17β-diol, and also by lower levels of the overall DS-R score and by a broad spectrum of steroids, both measured in the first trimester (Table 7). As for the role of the covariates, older

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women and those who had a longer pregnancy at the time of the measurement in the first trimester were significantly positively associated with higher levels of Δoverall DS-R score. This model explained 23.7% (19.3% after cross-validation) of Δoverall DS-R disgust score variability.

ΔCore disgust was significantly predicted by lower levels of overall DS-R score in the first trimester and by lower levels of steroids measured in the first trimester: 17-hydroxyprogesterone, 16α-hydroxyprogesterone, androstenedione, testosterone, 17-hydroxypregnanolone, 5α-androstane-3,17-dione, androsterone, androsterone sulfate, epiandrosterone sulfate, epietiocholanolone sulfate, 5α-androstane-3α,17β-diol, 5α-androstane-3β,17β-diol. This model explained 12.4% (9.9% after cross-validation) of ΔCore disgust score variability (Table 7).

The model for ΔContamination disgust revealed that higher levels of ΔContamination disgust were negatively associated with Δandrostenediol sulfate and also with many steroids measured in the first trimester (Table 7). Moreover, older women and those who had higher Δpregnancy length had higher ΔContamination disgust. The model for ΔContamination disgust explained 29.7% (24.9% after cross-validation) of ΔContamination disgust score variability.

In the model for ΔAnimal reminder disgust, higher Δdisgust was significantly predicted by higher levels of Δ5α-dihydrotestosterone, Δ5α-androstane-3α,17β-diol, Δpregnancy length, and again by lower levels of many steroids (e.g. testosterone, estradiol, androsterone) measured in the first trimester and also by lower levels of the Animal reminder disgust in the first trimester (Table 7). Moreover, women who had a longer pregnancy at the time of the measurement in the first trimester had significantly higher levels of ΔAnimal reminder disgust. This model explained 19.2% (15.3% after cross-validation) of ΔAnimal reminder score variability.

In the OPLS model for ΔPathogen disgust score of the TDDS, higher Δdisgust was significantly predicted by higher levels of pregnenolone (measured in the first trimester), Δestrone sulfate, Δestradiol sulfate, Δestriol sulfate. Moreover, lower levels of Pathogen disgust measured in the first trimester significantly predicted higher ΔPathogen disgust. This model explained 14% (11.2% after cross-validation) of ΔPathogen disgust score variability (Table 8). No covariate was associated with ΔPathogen disgust.

Table 7. Associations between Δdisgust sensitivity (trimester 3 - trimester 1) measured by the DS-R and predictors in the first trimester and Δpredictors evaluated by an OPLS model and multiple regression in pregnancy.

	OPLS (predictive component)						Multiple regression		
Variable	**Variable importance**	**t-statistics**		**Component loading**	**t-statistics**	**R[a]**	**Regression coefficient**	**t-statistics**	
ΔDS-R, Overall score									
Pregnenolone sulfate	0.771	2.97	*	-0.180	-8.45	-0.527 **	-0.021	-3.14	**
17-Hydroxypregnenolone sulfate	0.617	2.77	*	-0.162	-5.63	-0.470 **	-0.017	-2.69	*
16α-Hydroxypregnenolone	0.764	3.25	**	-0.194	-6.32	-0.569 **	-0.021	-3.56	**
DHEA sulfate	1.080	2.44	*	-0.207	-5.54	-0.605 **	-0.030	-2.85	*
7α-Hydroxy-DHEA	0.793	2.73	*	-0.237	-5.40	-0.693 **	-0.022	-3.93	**
7α-oxo-DHEA	0.642	2.71	*	-0.152	-10.42	-0.444 **	-0.018	-2.29	*
Androstenediol	0.641	3.62	**	-0.185	-6.52	-0.541 **	-0.018	-3.16	**
Androstenediol sulfate	0.852	4.59	**	-0.122	-3.62	-0.355 **	-0.023	-4.11	**
20α-Dihydroprogesterone	0.664	1.98	*	-0.092	-2.22	-0.268 *	-0.018	-1.53	
17-Hydroxyprogesterone	0.955	4.46	**	-0.146	-4.67	-0.428 **	-0.026	-3.39	**

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16α-Hydroxyprogesterone	1.179	6.49	**	-0.144	-4.17	-0.420	**	-0.032	-3.91 **
17,20α-Dihydroxy-4-pregnen-3-one	0.919	3.25	**	-0.179	-6.10	-0.527	**	-0.025	-2.61 *
Androstenedione	1.266	6.07	**	-0.227	-9.73	-0.663	**	-0.035	-3.75 **
Testosterone	1.416	9.51	**	-0.232	-8.69	-0.680	**	-0.039	-4.74 **
5α-Dihydrotestosterone	1.164	5.27	**	-0.244	-17.51	-0.712	**	-0.032	-4.52 **
Estrone	0.718	3.21	**	-0.094	-4.67	-0.265	**	-0.020	-4.78 **
Estradiol	0.779	2.92	*	-0.105	-4.24	-0.304	**	-0.021	-2.62 *
17α-Hydroxyallopregnanolone	1.242	3.34	**	-0.167	-4.75	-0.490	**	-0.034	-2.33 *
17α-Hydroxypregnanolone	1.305	3.71	**	-0.125	-2.41	-0.366	*	-0.036	-2.35 *
5β-Pregnane-3α,17,20α-triol	0.970	3.31	**	-0.140	-4.81	-0.412	**	-0.027	-2.44 *
5α-Androstane-3,17-dione	1.437	4.69	**	-0.233	-6.53	-0.682	**	-0.039	-3.48 **
Androsterone	1.552	12.86	**	-0.275	-15.44	-0.804	**	-0.042	-4.75 **
Androsterone sulfate	0.799	2.57	*	-0.134	-4.14	-0.391	**	-0.022	-2.89 *
Epiandrosterone	1.295	6.09	**	-0.260	-9.38	-0.761	**	-0.035	-4.87 **
Epiandrosterone sulfate	0.808	1.93	*	-0.151	-5.84	-0.441	**	-0.022	-2.19 *
5α-Androstane-3α,17β-diol	1.619	12.04	**	-0.260	-14.96	-0.762	**	-0.044	-6.40 **
5α-Androstane-3β,17β-diol	1.184	5.87	**	-0.192	-10.04	-0.561	**	-0.032	-7.80 **
Maternal age	0.692	2.67	*	0.125	3.89	0.364	**	0.019	2.08 *
Male sex of the fetus	0.790	2.16	*	-0.035	-1.26	-0.103		-0.022	-2.45 *
DSR_Overall score	0.851	3.18	**	-0.066	-2.62	-0.194	*	-0.023	-2.64 *
Pregnancy length	0.564	2.04	*	0.051	2.65	0.148	*	0.015	2.27 *
ΔDHEA sulfate	1.128	2.11	*	0.194	5.72	0.566	**	0.031	2.27 *
ΔAndrostenediol	0.857	2.49	*	0.156	4.55	0.458	**	0.023	2.07 *
Δ5α-Dihydrotestosterone	0.980	4.69	**	0.149	5.41	0.437	**	0.027	5.27 **
ΔAndrosterone	0.754	3.00	*	0.142	8.92	0.418	**	0.021	2.54 *
Δ5α-Androstane-3β,17β-diol	0.848	2.06	*	0.104	5.15	0.303	**	0.023	2.39 *
ΔAndrostenediol sulfate	0.608	2.34	*	0.010	0.31	0.029		0.017	2.31 *
ΔDS-R, Overall score				1.000	11.93	0.486	**		
Explained variability				23.7% (19.3% after cross-validation)					
ΔDS-R, Core disgust									
17-Hydroxyprogesterone	0.914	2.49	*	-0.257	-2.99	-0.519	*	-0.044	-1.82
16α-Hydroxyprogesterone	0.771	3.95	**	-0.203	-2.47	-0.410	*	-0.037	-2.45 *
Androstenedione	0.929	2.58	*	-0.361	-7.89	-0.729	**	-0.045	-2.12 *
Testosterone	0.886	11.65	**	-0.349	-14.24	-0.702	**	-0.043	-4.23 **
17-Hydroxypregnanolone	1.238	2.91	*	-0.220	-2.24	-0.446	*	-0.060	-2.07 *
5α-Androstane-3,17-dione	0.793	4.33	**	-0.359	-16.89	-0.724	**	-0.038	-2.82 *
Androsterone	0.862	4.60	**	-0.373	-7.41	-0.752	**	-0.042	-3.36 **
Androsterone sulfate	1.379	5.09	**	-0.288	-3.92	-0.573	**	-0.067	-7.75 **
Epiandrosterone sulfate	1.194	4.75	**	-0.295	-3.48	-0.587	**	-0.058	-10.59 **
Epietiocholanolone sulfate	0.769	2.34	*	-0.227	-2.75	-0.450	*	-0.037	-2.89 *
5α-Androstane-3α,17β-diol	1.068	2.74	*	-0.342	-6.91	-0.689	**	-0.052	-2.78 *
5α-Androstane-3β,17β-diol	0.839	4.42	**	-0.273	-4.22	-0.550	**	-0.041	-6.07 **
DS-R, Overall score	1.121	3.30	**	-0.154	-2.42	-0.312	*	-0.054	-3.09 **
ΔDS-R, Core disgust				1.000	2.80	0.352	*		
Explained variability				12.4% (9.9% after cross-validation)					
ΔDS-R, Contamination disgust									
Pregnenolone sulfate	0.695	2.53	*	-0.190	-10.55	-0.427	**	-0.031	-2.05 *
20α-Dihydropregnenolone	0.868	4.04	**	-0.145	-5.26	-0.325	**	-0.038	-4.67 **
DHEA sulfate	0.730	2.68	*	-0.205	-5.05	-0.460	**	-0.032	-3.03 **
Androstenediol	0.872	4.48	**	-0.207	-8.62	-0.468	**	-0.039	-5.64 **
Androstenediol sulfate	0.912	3.64	**	-0.141	-3.66	-0.316	**	-0.040	-2.66 *
Androstenedione	1.171	2.79	*	-0.280	-7.71	-0.633	**	-0.052	-3.31 **

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Testosterone	1.041	2.11	*	-0.278	-4.95	-0.628	**	-0.046	-2.26	*
Estrone	0.935	2.73	*	-0.186	-5.90	-0.399	**	-0.041	-2.63	*
Allopregnanolone	0.942	4.42	**	-0.172	-4.80	-0.389	**	-0.042	-4.26	**
Allopregnanolone sulfate	0.709	2.18	*	-0.073	-1.89	-0.164		-0.031	-1.85	
Isopregnanolone sulfate	0.785	1.90	*	-0.104	-2.76	-0.233	*	-0.035	-1.59	
17α-Hydroxypregnanolone	1.275	2.97	*	-0.199	-5.27	-0.448	**	-0.056	-3.48	**
5α,20α-Tetrahydroprogesterone	0.914	4.62	**	-0.164	-3.81	-0.372	**	-0.040	-3.60	**
5α-Pregnane-3α,20α-diol	0.632	4.27	**	-0.150	-4.82	-0.338	**	-0.028	-5.52	**
5β-Pregnane-3α,20α-diol	0.733	2.60	*	-0.150	-4.56	-0.340	**	-0.032	-2.89	*
5β-Pregnane-3β,20α-diol	0.900	4.13	**	-0.130	-3.59	-0.279	**	-0.040	-4.20	**
5β-Pregnane-3α,17α,20α-triol	0.967	2.73	*	-0.203	-5.01	-0.457	**	-0.043	-2.90	*
5α-Androstane-3,17-dione	1.274	5.03	**	-0.318	-13.20	-0.720	**	-0.056	-3.40	**
Androsterone	1.222	5.01	**	-0.319	-9.19	-0.720	**	-0.054	-5.36	**
Androsterone sulfate	1.384	6.74	**	-0.246	-6.37	-0.553	**	-0.061	-5.98	**
Epiandrosterone	0.761	3.80	**	-0.269	-17.69	-0.609	**	-0.034	-2.80	*
Epiandrosterone sulfate	1.181	4.83	**	-0.246	-8.24	-0.551	**	-0.052	-3.88	**
Etiocholanolone	0.818	2.29	*	-0.131	-2.69	-0.296	*	-0.036	-2.56	*
Conjugated 5α-androstane-3α,17β-diol	0.870	3.59	**	-0.151	-3.41	-0.336	**	-0.039	-3.15	**
Conjugated 5α-androstane-3β,17β-diol	0.874	2.10	*	-0.136	-3.15	-0.301	**	-0.039	-1.84	
Cortisone	1.256	3.77	**	-0.202	-4.02	-0.456	**	-0.056	-2.65	*
Maternal age	0.965	3.43	**	0.153	5.17	0.344	**	0.043	3.14	**
Pregnancy length	1.142	3.74	**	-0.077	-1.15	-0.172		-0.051	-3.04	**
ΔAndrostenediol sulfate	1.477	18.03	**	-0.133	-6.36	-0.298	**	-0.065	-7.19	**
ΔPregnancy length	0.982	3.15	**	0.081	2.37	0.182	*	0.043	2.69	*
ΔDS-R, Contamination disgust				1.000	11.03	0.545	**			
Explained variability				29.7% (24.9% after cross-validation)						
ΔDS-R, Animal reminder disgust										
7-oxo-DHEA	0.837	2.81	*	-0.165	-4.43	-0.423	**	-0.029	-3.41	**
20α-Dihydroprogesterone	1.210	3.93	**	-0.238	-4.74	-0.608	**	-0.042	-4.37	**
16α-Hydroxyprogesterone	1.150	5.03	**	-0.248	-6.41	-0.634	**	-0.040	-5.40	**
17α,20α-Dihydroxy-4-pregnene-3-one	0.851	2.17	*	-0.239	-6.31	-0.612	**	-0.030	-2.95	*
Testosterone	0.990	3.34	**	-0.271	-13.71	-0.692	**	-0.035	-3.99	**
5α-Dihydrotestosterone	0.979	4.33	**	-0.258	-5.93	-0.660	**	-0.034	-3.38	**
Estradiol	0.844	2.62	*	-0.164	-3.95	-0.414	**	-0.030	-2.50	*
17α-Hydroxyallopregnanolone	0.898	4.74	**	-0.263	-11.02	-0.674	**	-0.031	-3.03	**
17α-Hydroxypregnanolone	0.981	3.14	**	-0.210	-5.96	-0.539	**	-0.034	-2.82	*
3α,5α-Tetrahydroprogsterone	1.042	2.61	*	-0.250	-8.76	-0.639	**	-0.036	-2.59	*
5α-Pregnane-3α,20α-diol	0.840	2.34	*	-0.248	-5.20	-0.635	**	-0.029	-2.52	*
3α,5β-Tetrahydroprogsterone	1.446	3.89	**	-0.204	-7.26	-0.521	**	-0.051	-2.67	*
5β-Pregnane-3α,20α-diol	1.146	3.78	**	-0.204	-12.24	-0.521	**	-0.040	-2.69	*
5β-Pregnane-3α,17α,20α-triol	0.774	4.00	**	-0.212	-8.23	-0.545	**	-0.027	-3.78	**
Androsterone	0.972	11.39	**	-0.273	-7.07	-0.699	**	-0.034	-6.20	**
Epiandrosterone	1.023	3.51	**	-0.215	-4.07	-0.550	**	-0.036	-2.94	*
5α-Androstane-3α,17β-diol	1.231	3.52	**	-0.262	-6.79	-0.670	**	-0.043	-2.91	*
5α-Androstane-3β,17β-diol	1.148	1.93	*	-0.217	-3.69	-0.554	**	-0.040	-1.79	
Parity	0.908	3.68	**	0.081	1.60	0.206		0.032	2.84	*
DS-R, Animal reminder disgust	0.944	3.23	**	-0.080	-2.67	-0.205	*	-0.033	-2.36	*
Pregnancy length	0.716	2.65	*	0.114	3.14	0.292	**	0.025	3.18	**
Δ5α-Dihydrotestosterone	0.714	3.04	**	0.204	5.83	0.523	**	0.025	2.76	*
Δ5α-Androstane-3α,17β-diol	1.013	3.00	**	0.186	9.32	0.477	**	0.035	2.32	*
ΔPregnancy length	0.993	2.67	*	0.102	2.13	0.261	*	0.035	3.09	**
ΔDS-R, Animal reminder disgust				1.000	20.08	0.438	**			

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Explained variability	19.2% (15.3% after cross-validation)

[a]R…Component loadings expressed as correlation coefficients with predictive component, *p<0.05, **p<0.01.

Table 8. Associations between ΔPathogen disgust (trimester 3 - trimester 1) measured by the TDDS and predictors in the first trimester and Δpredictors evaluated by an OPLS model and multiple regression in pregnancy.

	OPLS, predictive component						Multiple regression	
Variable	**Variable importance**	**t-statistics**		**Component loading**	**t-statistics**	**R[a]**	**Regression coefficient**	**t-statistics**
Pregnenolone	0.642	2.83	*	0.183	1.98	0.259 *	0.075	1.98 *
TDDS_PATHOGEN	1.001	4.05	**	-0.333	-4.28	-0.474 **	-0.118	-4.83 **
ΔEstrone sulfate	1.140	3.91	**	0.517	10.61	0.740 **	0.134	2.92 *
ΔEstradiol sulfate	1.061	3.80	**	0.566	16.62	0.804 **	0.125	2.67 *
ΔEstriol sulfate	1.077	9.86	**	0.553	14.69	0.804 **	0.127	6.58 **
ΔPathogen disgust (trimester 3 - trimester 1)				1.000	2.72	0.375 *		
Explained variability	14% (11.2% after cross-validation)							

[a]R…Component loadings expressed as correlation coefficients with predictive component, *p<0.05, **p<0.01.

3. Discussion

In order to better understand the physiological mechanisms involved in the changes in disgust sensitivity during pregnancy, we have investigated associations between a broad spectrum of steroids and disgust sensitivity in the first and third trimesters of pregnancy. In view of the complexity of the study, let us first summarize and discuss associations between the studied steroids and disgust sensitivity which were observed in both the first and the third trimester while leaving aside, for the moment, the questionnaire used for measuring disgust sensitivity and the individual disgust subscales. Then we will focus on the specific associations observed in each trimester separately and discuss the steroid changes accompanying the shift in disgust sensitivity between the first and the third trimester. In the final part of this section, we discuss the specifics of selected disgust subscales and differences between the results in disgust sensitivity obtained by the two questionnaires (DS-R and TDDS) we have used.

In both trimesters, we found mainly positive correlations between disgust sensitivity and C19 steroids (androgens), including testosterone and 5α-dihydrotestosterone as active androgens, but also androstenediol and DHEA or their sulfates as the precursors of active androgens in the Δ^5 pathway, and androstenedione as a precursor in the Δ^4 pathway. C19 steroid levels reflect above all the activity of maternal adrenal zona reticularis. Although these positive associations were observed in both trimesters, they were significantly more pronounced in the first trimester for C19 Δ^4 steroids and in the third trimester for C19 Δ^5 steroids. In both trimesters, we have also observed positive correlations between disgust sensitivity and 5α/β-reduced metabolites of the C19 steroids. In the first trimester, we found a positive association between disgust sensitivity, 17-hydroxypregnanolone, and estrogens such as estradiol and estrone (with the exception of the DS-R overall score, where we found a negative correlation with estrone). In the third trimester, we found a positive association between disgust sensitivity and cortisol. To summarize: we have confirmed our initial hypotheses and found that disgust sensitivity positively correlates with the levels of steroids with immunomodulatory effects, such as testosterone, cortisol, estradiol, or 7α/β-hydroxy-, 7-oxo-derivatives of adrenal

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androgens, DHEA, and androstenediol (e.g. [42,43]). We have also confirmed the predicted association between disgust sensitivity and steroids such as estrogens, testosterone, cortisol, 7α-, 7β- and 16α-hydroxy-metabolites of C19 Δ^5 steroids, and 5α/β-reduced pregnane steroids, which were observed to be associated with mental wellbeing and certain mental disorders (e.g. [43,46]).

Focusing now on results pertaining to the first trimester of pregnancy, we have observed a positive correlation between disgust sensitivity (specifically the Contamination and Animal reminder subscales of the DS-R and the Pathogen domain of the TDDS) with estrogens (estrone and estradiol), whose production in pregnancy depends on the production of C19 Δ^5 steroid sulfates (DHEA sulfate, androstenediol sulfate) in the fetal zone of the fetal adrenal gland [47]. In the first trimester, the levels of DHEA sulfate also positively correlated with the scores of the Contamination disgust subscale of the DS-R. Our results are not in line with two studies that found no association between salivary estradiol levels and disgust sensitivity measured by TDDS [18,29], but it should be noted that both of the aforementioned studies were conducted on a population of nonpregnant women and they focused on the relationship between disgust and estradiol levels during the menstrual cycle. On the other hand, some studies have shown higher levels of disgust sensitivity during the luteal phase of the menstrual cycle, when estrogen levels are elevated, in comparison with the menstrual phase [20,23]. That is in line with our findings. Similarly to the immunosuppressive effect of progesterone, which inspired the formulation of the compensatory prophylaxis hypothesis [13], estrogens, too, play a role in immunomodulation. They are known to shift the immune response towards Th2 dominance [48].

Nevertheless, our results regarding estrone levels are not entirely unambiguous. While higher estrone levels were associated with higher scores in the Contamination and Animal reminder disgust subscales of the DS-R and the Pathogen disgust domain of TDDS, the overall DS-R score correlated with estrone levels negatively. It must be taken into consideration that aside from the Contamination and Animal reminder disgust subscales, the DS-R also contains a Core disgust subscale, focused on food and animal or bodily products, which also contributes to the overall score. This subscale may have been the cause of the observed reverse direction of correlation. A better understanding of the observed effects would, however, require further research.

In addition to the substances mentioned above, we have also found positive correlations between disgust sensitivity in the first trimester and the levels of some C21 5α/β-reduced steroids, such as conjugated pregnanolone, 17-hydroxypregnanolone, 5α,20α-tetrahydroprogesterone, 5β,20α-tetrahydroprogesterone, 5β-Pregnane-3α,17α,20α-triol, and 5α-androstane-3,17-dione. Regarding the origin of various types of maternal steroids, it is known that the C21 steroids in pregnancy owe their origin mainly to placental progesterone, which may be a product of LDL cholesterol penetrating from the maternal compartment into the placenta, but also to pregnenolone sulfate, which is formed in the fetal adrenal gland and then metabolized to progesterone in the placenta. Maternal C19 steroids (discussed below) mostly originate in the zona reticularis of the maternal adrenal cortex and their levels depend on the activity of the enzyme membrane-bound hemoprotein CYB5 [49]. All this points to an important role of maternal and placental steroidogenesis in mother's susceptibility to disgust.

In connection with the C21 5α/β-reduced steroids, 5α-androstane-3,17-dione deserves a separate mention. The levels of this steroid consistently positively correlated not only with disgust levels as measured by the DS-R and TDDS questionnaires in the first trimester but also with disgust sensitivity measured using DS-R in the third trimester of pregnancy. While not bioactive itself, 5α-androstane-3,17-dione is a precursor of 5α- androstandiols. Among this group, 5α-androstanols that have a hydroxyl group in the 3α position and are not conjugated – such as androsterone, etiocholanolone, and 5α-androstane-3α,17β-diol – act as positive GABAergic (neuroinhibitory) modulators. The biologically inactive 5α-androstane-3,17-dione is a direct 5α-reduced metabolite of the likewise biologically inactive androstenedione, which is a direct precursor of both the biologically active androgen testosterone and the biologically inactive estrone. Estrone, however, is further metabolized to create the active estrogen estradiol. In addition, the 5α-androstane-3,17-dione can be readily converted to the most active androgen 5α-dihydrotestosterone in a single metabolic step (by

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reduction of the oxo-group to 17beta-hydroxy-group), and this conversion works in both directions [50]. Higher levels of 5α-androstane-3,17-dione can therefore indicate either a higher production of active sex steroids or, conversely, their higher catabolism. Considering that the results discussed below show a significant positive association between active sex steroids and disgust sensitivity, it would seem that in this case, the elevated levels of 5α-androstane-3,17-dione are more likely to be related to a higher production of these steroids.

In both trimesters, we have also found a significant positive correlation between disgust sensitivity and 5α-dihydrotestosterone. A significant positive correlation with testosterone levels was observed only in the first trimester; the sole exception was the Contamination disgust subscale of DS-R, where we observed an increase in scores in association with decreasing testosterone levels (this relationship is discussed separately below, in connection with specific outcomes related to this subscale). Because higher testosterone levels are associated with increased immunosuppression [51], our main findings are in line with the compensatory prophylaxis hypothesis [13], which suggests that during immunosuppression, disgust sensitivity should be elevated. Moreover, higher disgust sensitivity has been observed in women pregnant with male as opposed to female fetus [10,30], which may reflect possible mechanisms leading to elevated testosterone levels in women pregnant with a male fetus [52]. On the other hand, studies that focused on relationships between the levels of salivary testosterone and disgust sensitivity in nonpregnant female populations so far found no significant association between the two [18,29]. Furthermore, our study provides no support for a potential influence of the sex of the fetus on disgust sensitivity during pregnancy: fetus sex did not statistically significantly contribute to the overall model in any of the conducted analyses.

Contrary to our expectations, we did not find any significant correlation between disgust sensitivity and the most active immunomodulatory 7α/β-hydroxy-, 7-oxo-, and 16α-hydroxy-metabolites (e.g., 5-androstene-3β,16α, and17β-triol and its sulfate) of C19 Δ^5 steroids in the first trimester. Recent studies have reported that the first trimester is a time of significant immunomodulation [31,32]. Additionally, in line with the compensatory prophylaxis hypothesis [9,13], it has been demonstrated that disgust sensitivity negatively correlates with certain cytokines [34] and, similarly, that disgust sensitivity is elevated in the first trimester in women who were recently ill [10]. We have therefore expected some significant relationships between disgust sensitivity and a number of steroids with immunomodulatory function during this period of pregnancy as well. We found, however, a positive association between disgust sensitivity and androstenediol (a C19 Δ^5 steroid) during this period of pregnancy, whereby androstenediol belongs to adrenal androgens, hormones produced by adrenal glands which are precursors to, among others, testosterone and estrogen, two sex hormones for which we found a positive correlation with disgust sensitivity. Moreover – as will be discussed in more detail just below – androstenediol also reflects immunomodulatory processes in pregnancy.

The aforementioned immunoreactive metabolites of C19 Δ^5 androstanes became more prominent in correlations with disgust sensitivity (measured by both the DS-R and TDDS) during the third trimester. Positive associations were found for DHEA, 7α-hydroxy-DHEA, 7β-hydroxy-DHEA, 5-Androstene-3β,7α,17β-triol, and androstenediol. While these substances stimulate immune response, they also suppress autoimmunity. On one hand, C19 Δ^5 steroids (including the aforementioned metabolites) mitigate the severity of autoimmune diseases [53–58] but on the other hand, autoimmune diseases can weaken the production of adrenal C19 Δ^5 steroids [53,59]. Some of these steroids may also counteract the suppression of the primary immune response by glucocorticoids [60]. It has been also reported that DHEA controls the Th1/Th2 balance and either favors the Th1 component or attenuates the production of both components [57,61]. C19 Δ^5 steroids also suppress cell-mediated immunity and the formation of autoantibodies [55–58,62], and they may induce restoration of the Th1-dominated cytokine profile. The mechanism explaining the immunomodulatory effects of the 7α/β- Δ^5-steroids may be associated with a competition of 7-oxygenated androstanes for the active sites on the HSD11B1, which catalyzes the conversion of inactive 11-oxo-glucocorticoids to their immunosuppressive 11β-hydroxy-counterparts [63,64].

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The autoimmune response can also be induced by estradiol, specifically via estrogen receptors, which is another mechanism of C19 Δ^5 steroid action associated with the catabolism of C19 estrogen precursors such as DHEA, androstenediol, and 5α-androstane-3β,17β-diol (which are also estrogenic) to their 7-oxygenated and 16α-hydroxylated catabolites that cannot be further converted to bioactive estrogens [65]. Interestingly, estradiol can stimulate catalytic CYP7B1 activity, mRNA, and human CYP7B1 reporter gene in human embryonic kidney cells HEK293, and it may control the DHEA, estradiol, and androstenediol levels in human tissues [66]. 5-androstene-3β,7β,17β-triol, which may be either formed by interconversion from 5-androstene-3β,7α,17β-triol, or directly from androstenediol by the catalytic action of CYP3A4 and CYP3A7, is immunoprotective despite low concentrations and high clearance [67]. Synthetic anti-inflammatory derivatives of 5-androstene-3β,7β,17β-triol suppress the production of C-reactive protein interleukin 17 (IL-17), TNFα, interleukin 6 (IL-6) signaling, and the expression of mRNA for IL-6 and matrix metalloproteinase in inflamed tissues. These steroids also suppress pro-inflammatory cytokines in the lungs and intensely stimulate splenic regulatory T-cells [68].

To summarize, the elevated levels of C19 Δ^5 steroids during the third trimester of pregnancy could reflect an adaptive mechanism of response to the increasing need for protection against pathogens with approaching childbirth. After birth, the newborn infant is extremely vulnerable and since its own immune system is not yet developed, it relies primarily on maternal protection against infections.

In addition to the C19 Δ^5 androstanes, cortisol, and some steroids in the metabolic pathway of cortisol synthesis, such as pregnenolone and 17-hydroxypregnenolone, are also positively associated with disgust sensitivity as measured by the DS-R questionnaire in the third trimester, which may be of interest given the well-known immunosuppressive effect of cortisol [69]. Moreover, when compared with a control group of women, higher levels of cortisol, as well as higher levels of DHEA (discussed above), have also been observed in female patients with obsessive-compulsive disorder [46]. This is consistent with the observed positive association between disgust and anxiety-related disorders, including the obsessive-compulsive disorder [12,70,71], and also in line with the specific results obtained for the Contamination disgust subscale of the DS-R questionnaire.

In contrast to all other findings regarding disgust sensitivity and steroids, a lower level of testosterone in the third trimester predicted higher disgust scores in the Contamination subscale of DS-R. It is also important to note that the testosterone levels had the strongest effect in this particular model: they explained 32.5% of variability. Similar to cortisol and DHEA, which were previously associated with anxiety disorders, lower salivary testosterone levels were measured in women with current depressive disorder, generalized anxiety disorder, social phobia, and agoraphobia without panic disorder [72]. That indicates that the increased disgust sensitivity during pregnancy need not be only an adaptive mechanism aimed at protecting the organism from pathogens. In the third trimester, it may also reflect the higher anxiety observed during this period [38,73], which may be associated with the approaching childbirth. The Contamination disgust subscale of the DS-R is centered around worries about interpersonal transmission of pathogens and subsequent aversion. Contamination disgust is closely related to some types of the obsessive-compulsive syndrome, whose symptoms include compulsive cleaning and handwashing. Our findings are also consistent with the study by Dlouhá et al. [10], where the authors observed increasing levels of disgust sensitivity during pregnancy that extended into the postpartum period.

Besides a negative correlation between testosterone levels and scores on the Contamination disgust subscale, we have also found positive correlations between Contamination disgust scores in the third trimester and a number of progesterone metabolites such as conjugated pregnanolone, conjugated epipregnanolone, conjugated 5α,20α-tetrahydroprogesterone, conjugated 5α-pregnane-3α,20α-diol, conjugated 5β,20α-tetrahydroprogesterone, and conjugated 5β-pregnane-3β,20α-diol. These relationships may be related to placental production of progesterone. Given that progesterone can be rapidly metabolized, these catabolites may be more stable markers of its presence, which could explain the absence of progesterone itself in these relationships. If this is indeed the case, it would be in line with the assumption of the compensatory prophylaxis hypothesis according to which the

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immunosuppressive function of progesterone is compensated by elevated disgust sensitivity: this has been previously shown in two studies on nonpregnant, naturally cycling women [20,24] and in an animal model [28]. A positive association was also observed between progesterone and increased sensitivity to disgusted faces with averted gaze, which might signal a pathogen threat in the environment [25].

Regarding the correlations between changes in disgust sensitivity and increasing pregnancy length, there was a general trend towards negative correlations between increased disgust sensitivity and the overall activity of steroidogenesis during the first trimester of pregnancy. We have also observed positive correlations between disgust sensitivity and the increase in C19 steroid levels between the first and third trimesters. This shows that, on one hand, lower steroidogenic activity in the first trimester is associated with a more significant increase in disgust sensitivity with increasing pregnancy length due to increased steroid production, and on the other hand, it confirms the positive association between zona reticularis activity in the maternal adrenal gland and disgust sensitivity. The role of the fetus is not decisive here, because although the fetal zone of the fetal adrenal gland produces even more sulfated Δ^5 androstanes than the zona reticularis in the maternal adrenal gland, these substances are rapidly metabolized to estrogens in the placenta, so that while maternal blood estrogen levels increase exponentially with advancing gestational age, maternal adrenal androgen levels do not [47].

Associations between specific steroid hormones and the two different questionnaires, the DS-R and the Pathogen domain of TDDS, allow us to make some observations about the questionnaires themselves. The Pathogen domain of TDDS is often associated with similar steroids as the Core and Animal reminder subscales of the DS-R. During the first trimester, only a minimum of steroids is specifically associated only with the Pathogen domain, which suggests that the Pathogen domain reflects some part of the disgust sensitivity measured by the DS-R. The DS-R has been previously criticized for not effectively reflecting the adaptive function of disgust. Based on this critique, the TDDS questionnaire was developed to focus specifically on the adaptiveness of disgust sensitivity [45]. The results obtained from the two different questionnaires would suggest that the DS-R actually reflects both the adaptive function of disgust and the maladaptive form of disgust associated with, for instance, anxiety disorders.

Strengths and Limitations

Our study has several strengths. Due to its longitudinal design, we were able to track the development of disgust levels in response to changes in steroid levels during pregnancy. Moreover, we had a relatively large sample of women, which is rather uncommon for this type of study. Another strength of the study is the simultaneous use of two of the most commonly used textual questionnaires (DS-R and TDDS) to measure disgust sensitivity. The inclusion of both questionnaires enabled us to better understand the various aspects of disgust and to detect overlaps and differences between the questionnaires.

The main limitation of this study is that disgust sensitivity was based on self-report textual questionnaires. Aside from textual questionnaires, there are other methods of measuring disgust sensitivity, for instance those based on visual stimuli. Such methods can also allow for measurement of the experienced emotion based on various physiological parameters that directly reflect the subject's state. In the case of pregnant women, however, we could not use such methods for ethical reasons. Nevertheless, some studies used the textual and visual methods in parallel and found no differences in the results acquired by the two approaches [16].

4. Methods

In a prospective longitudinal study running between June 2019 and November 2022, we have collected data from pregnant women in the first and the third trimester of pregnancy in collaboration with three private gynecological clinics in Prague, Czech Republic. This study was a part of a larger project to explore longitudinal changes in pregnancy and their correlations with biological and psychological factors.

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4.1. Procedure – Data Collection

In total, 228 adult women who conceived naturally and reported no reported severe chronic diseases or autoimmune disorders were recruited for the study in collaboration with three gynecological clinics in Prague, Czech Republic. From this sample, 49 women were excluded from the study: 15 women miscarried, 19 women either did not provide blood samples in both trimesters or there was insufficient amount of blood serum for steroid hormone testing, and 15 women left the study at their request. The final sample thus consisted of 179 women.

Participants were recruited for the study during their first antenatal medical checkup where their pregnancy was confirmed by their gynecologist. At this time, between week 5 and 14 of pregnancy (mean ± SD = 7.7 ± 1.23), they completed a background questionnaire which included questions about age, physical parameters, parity, the method of conception, health status, and several demographic questions. During medical checkups during the first trimester, between weeks 9 and 14 of pregnancy (mean ± SD = 10.3 ± 0.91) and again during the third trimester, between weeks 30 and 38 pregnancy (mean ± SD = 33.0 ± 1.61), they completed questionnaires that measured disgust sensitivity and provided blood samples for determining the levels of steroid hormones. Information about the sex of the baby was obtained from both medical records and questionnaires.

All women participating in this study were part of a larger study focused on prenatal factors that affect the mother's and child's wellbeing and health. Participants answered all the disgust questions along with pregnancy nausea questions and were not specifically informed that the study was about disgust sensitivity, because that could influence their responses. All women signed an informed consent and participated in all parts of the study under a pseudo-anonymous code. The project was approved by the Institutional Review Board of the Faculty of Science, Charles University (Approval No. 2018/6 and 2019/10). All methods were performed in accordance with the relevant guidelines and regulations.

4.2. Questionnaires

All women completed a background questionnaire, including information about age, history of previous pregnancies, education level, size of residence area, and health data. Disgust sensitivity was assessed by the Disgust Scale-Revised (DS-R) [44] and by the Pathogen domain of the Three Domains of Disgust Scale (TDDS) [45].

The DS-R [44] is a 25-item self-report inventory consisting of three subscales: Core disgust subscale (12 items; disgust elicited by food and animal or bodily products), Animal reminder disgust subscale (8 items; disgust related to mortality, possible injuries, or body envelope violations), and Contamination disgust subscale (5 items; disgust related to the interpersonal transmission of pathogens). There are five possible responses to each item ranging from 0 to 4. In the first part of the DS-R (13 items), respondents rate how much they agree with given statements, or how true the statements are about them: "0 = Strongly disagree (very untrue about me), 1 = Mildly disagree (somewhat untrue about me), 2 = Neither agree nor disagree, 3 = Mildly agree (somewhat true about me), 4 = Strongly agree (very true about me)". In the second part of the questionnaire (12 items), the respondents rate how disgusting they would find described experiences: "0 = Not disgusting at all, 1 = Slightly disgusting, 2 = Moderately disgusting, 3 = Very disgusting, 4 = Extremely disgusting". The overall score may range from 0 to 100 (Core subscale from 0 to 48, Animal reminder subscale from 0 to 32, and Contamination subscale from 0 to 20). A higher score indicates greater disgust sensitivity. If one-fifth or fewer responses were missing for each subscale, we used the average score of the corresponding subscale to supplement the missing values (we supplemented seven responses in the first trimester and five responses in the third trimester). Participants with more than one-fifth of the items unanswered were excluded from the analyses (ten in the first trimester and three in the third trimester).

The TDDS [45] is a 21-item self-report inventory with three domains (Pathogen, Moral, and Sexual). For our study, we used just the 7-item Pathogen domain which is the most relevant for the current investigation and to avoid overload of the participants. Respondents rate the statements from 0 to 6, where 0 = not disgusting at all and 6 = extremely disgusting. The score of the Pathogen domain

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may thus range from 0 to 42. If one-fifth or fewer responses were missing, we used the average score to supplement the missing values (we supplemented one response in the first trimester and two responses in the third trimester). Participants with more than one-fifth of the items unanswered were excluded from the analyses (five in the first trimester and three in the third trimester).

4.3. Laboratory Measurement of Steroid Hormones

Blood samples of 179 women (the final sample) in the 1st and 3rd trimester of pregnancy were analyzed for concentrations of steroid hormones in blood serum. Serum from blood was obtained after centrifugation (2 min at 3,000 × g at 21 °C), and stored at -20 °C until analyzed. We used the advanced GC-MS/MS platform for the multicomponent quantification of endogenous steroids, 56 unconjugated steroids and 35 polar conjugates of steroids (after hydrolysis). Unlike the current methods used for the quantification of circulating steroids on the GC-MS/MS platform, the present one was validated not only for the blood of men and non-pregnant women but also for the blood of pregnant women and for mixed umbilical cord blood [74]. The spectrum of analytes includes common hormones operating via nuclear receptors as well as other bioactive substances like immunomodulatory and neuroactive steroids. The present method was extended for corticoids and 17-hydroxylated 5α/β-reduced pregnanes, which are useful for the investigation of an alternative "backdoor" pathway. The testing was carried out in the Institute of Endocrinology under the direction of Martin Hill, PhD, DSc.

4.4. Statistics

At first, the data were both manually and automatically controlled. Before conducting the final statistical analyses, all variables, except for binary variables, were transformed towards symmetric distribution and constant variance using STATGRAPHICS Centurion 18 software (The Plains, VA, USA). The values of all variables including steroids were transformed by power transformations to attain symmetric data distribution and homoscedasticity; the transformed data were automatically converted to z-scores by SIMCA software for further analyses.

Multivariate regression with a reduction of dimensionality known as orthogonal projections to latent structures (OPLS) [75] was conducted to assess the associations between hormonal changes and changes in disgust sensitivity during pregnancy. The OPLS model searches for the best linear combination of predictors for an optimum estimate of the dependent variable. Statistical software SIMCA v. 12.1.1.1. (Umetrics, Umea, Sweden) was used for the OPLS analysis.

In the input models, disgust sensitivity was represented by vector Y. Steroid levels and related variables, namely maternal age, maternal pre-pregnancy BMI (calculated based on a self-report of the participant's pre-pregnancy weight and height), pregnancy length in the first and third trimester (in days), maternal weight gain (in kg), parity (0: primipara, 1: multipara), fetus sex (0: female, 1: male), maternal diabetes and hypertension (0: no, 1: yes), maternal pre-pregnancy smoking (1: no, 2: only occasionally, 3: yes) constituted matrix X. Separate OPLS models were developed for the associations measured in the first trimester, in the third trimester and also for the changes between the first and third trimester (association between delta scores of disgust sensitivity and Δ(steroids) calculated as the levels of steroids/disgust scores in the third trimester minus the levels of steroids/disgust scores in the first trimester). In all cases, separate OPLS models were also developed for the Pathogen disgust measured by the TDDS, the overall DS-R score and the three individual DS-R subscales. By using this approach, one predictive component was extracted for each model. Non-homogeneities were eliminated after checking the data homogeneity in predictors using Hotelling's statistic.

5. Conclusions

While in the first trimester disgust increases with rising maternal estrogen levels and C19 Δ^5 steroids action, which are associated with catabolism of C19 estrogen precursors, in the third trimester, more positive correlations dominate between disgust levels and steroids, reflecting both maternal immune activity and potential symptoms accompanying anxiety or other psychiatric

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disorders. Understanding the neural, cognitive, and behavioral intricacies of the disgust system together with their associations with physiological mechanisms not only broadens our comprehension of human adaptive mechanisms but also holds implications for a diversity of fields, ranging from psychology and neuroscience to public health. Especially crucial is the understanding of these relationships during pregnancy, which represents a sensitive period for the mother and can significantly influence the future development of her child.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Review Board of the Faculty of Science, Charles University (Approval No. 2018/6 and 2019/10).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to privacy restrictions.

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