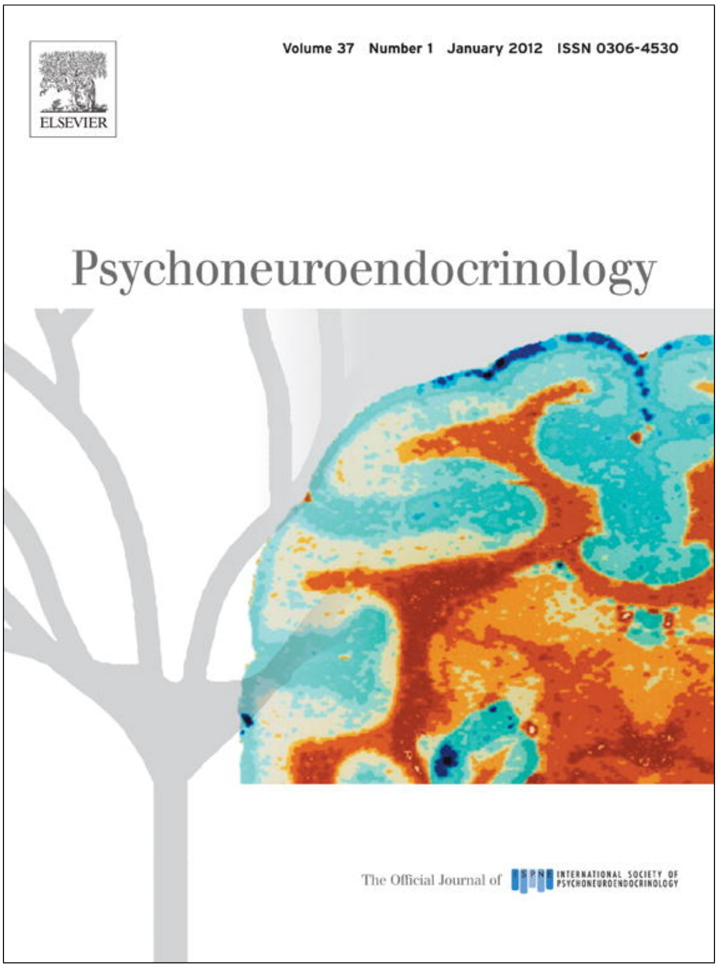
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# Cortisol and anxiety response to a relaxing intervention on pregnant women awaiting amniocentesis

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**KEYWORDS**
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**Summary**
*Background:* Stress and anxiety during pregnancy have been associated with premature and low birth weight babies, presumably through fetus over exposion to glucocorticoids. Antenatal stress also seems to have long-term effects upon infant development and adult health. However, medication for stress may carry risks to the expectant mother, therefore the efficacy of non-pharmacological interventions should be investigated.
*Methods:* Pregnant women ($n = 154$) awaiting amniocentesis, were randomly assigned in the morning and the afternoon to three groups for 30 min: (1) listening to relaxing music, (2) sitting and reading magazines, and (3) sitting in the waiting-room. Before and after that period, they completed the Spielberger's State and Trait anxiety inventory and provided blood samples for cortisol. The groups were then compared regarding change in cortisol levels and anxiety.
*Results:* Maternal cortisol and state anxiety were correlated ($r = 0.25$, $p = 0.04$) in the afternoon, but not in the morning. The larger decreases in cortisol occurred in the music group (−61.8 nmol/L, ANOVA: $p = 0.01$), followed by magazine, being differences among groups more pronounced in the morning. Women in the music group also exhibited the greater decreases in state anxiety ($p < 0.001$). Younger mothers with less gestational age were on average the most anxious, and also the ones with greater decreases in cortisol and anxiety levels after relaxation.
*Conclusion:* A relaxing intervention as short as 30 min, especially listening to music, decreases plasma cortisol and self-reported state anxiety score. Pregnant women might benefit from the routine practice of relaxation in the imminence of clinical stressful events.


## 1. Introduction

Accumulated evidence suggests that prenatal stress and anxiety experienced by expectant mothers is associated with pregnancy outcome and with long-lasting behavioural and

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cognitive effects in the child. Women with high antenatal anxiety or stress are more likely to have premature and low birth weight babies (Copper et al., 1996; Wadhwa et al., 1993, 1998; Field et al., 2003). Prenatal maternal stress is also suspect of affecting infant development and promoting a range of adverse temperamental effects (O'Connor et al., 2002, 2003; Field et al., 2003; Huizink et al., 2004; Laplante et al., 2004; Van den Bergh et al., 2005; Talge et al., 2007; Glover et al., 2010). The most widely exploited hypothesis for the underlying mechanisms states that the fetus is exposed to an excess of maternally derived glucocorticoids when the latter are raised at times of stress. Such fetal exposure underpins alterations in brain development and in the functioning of the hypothalamic—pituitary—adrenal axis (HPA) (Van den Bergh et al., 2005; Cottrell and Seckl, 2009; Harris and Seckl, 2011; Charil et al., 2010; Glover et al., 2010).

When psychological problems arise during pregnancy, an assessment of the medication risks to the mother and the fetus must be made, since antidepressants and benzodiazepines are known to pass across the placenta (Hendrick et al., 2003). Non-pharmacological therapies should be considered as possible choices for pregnant women attempting to avoid medication side-effects. These include psychotherapy, massage therapy, yoga, and listening to music (Cunningham and Zayas, 2002; Narendran et al., 2005; Chang et al., 2008; Kimber et al., 2008; Maharana et al., 2009; Field et al., 2009, 2010), the latter being probably the most accessible non-pharmacological therapy for low socioeconomic status women. Yoga, also a potentially accessible therapy, reduces sympathetic activity improving autonomic responses to stress in normal pregnant women (Maharana et al., 2009) but its practice is not well accepted in all cultures. A number of authors have discussed and reviewed the uses of music therapy to positively affect physiological functioning (Hirsch and Meckes, 2000; Schneck and Berger, 2006; Krout, 2007) and its relevance seems to be changing, being now considered more of a neuroscience than a social science model (Thaut, 2005). Music is a powerful tool in evoking emotions and, by extension, listening to music can be explored for reducing the negative effects of stress. Supportive evidence can be found in the observation that listening to music resulted in a marked reduction in salivary cortisol levels in patients exposed to pre-surgical stress, both in a medical (Miluk-Kolasa et al., 1994) and in a nonmedical setting (Khalfa et al., 2003). These experiments suggested that relaxing music is more effective than silence in decreasing cortisol levels after stress induction. Further studies are necessary to determine if the effect of relaxing music is specific to music or can be obtained with different stimuli.

A randomized controlled trial was conducted to test the hypothesis that music decreases anxiety and plasma cortisol levels in pregnant women subjected to stressful situations. Imminent amniocentesis was taken as a non-experimental paradigm for such a situation because it has been reported that state anxiety, a transient emotional condition, is considerably elevated in pregnant women awaiting an invasive procedure like amniocentesis and this can lead to an increase in their plasma cortisol (Harris et al., 2004; Ng et al., 2004; Sarkar et al., 2006, 2008).

## 2. Methods

This study was conducted in Lisbon, between December 2009 and November 2010, at Dona Estefânia Hospital (HDE) maternity, and involved pregnant women attending the routine appointment on prenatal diagnosis and amniocentesis briefing. By the end of the appointment, women without exclusion criteria were informed that our study was taking place, its basic procedures, and asked if willing to participate. Only singleton spontaneous pregnancies were considered, with other exclusion criteria being fetal structural abnormalities and those later associated with fetal aneuploidy or fetal death. Women without sufficient Portuguese language, phobic to needles, with a known underlying medical condition or under any prescribed medication were also excluded. Scheduling for amniocentesis was done already with an indication on whether they would participate. A total of 157 women with gestational age between 110 and 165 days were recruited. The gestational age was previously determined to the nearest day based on ultrasound biometry.

Amniocentesis took place between 8:30 h and 15:00 h. At arrival, after written informed consent to participate, as approved by the HDE ethics committee, we have collected information on demographic covariates and measured pulse and blood pressure. Women also filled-in a self-evaluation questionnaire on anxiety, followed by blood sample collection for cortisol. In order to evaluate the hormonal and psychological impact of a non-pharmacological intervention in a clinical setting, they were then randomly assigned to three different groups for 30 min — a group seated with the lights on while listening to relaxing music from 15 W loudspeakers with volume regulated according to the participant's will (range of 45—60 dB). A second group relaxed by sitting and reading decoration magazines in silence, and a third group simply waited in the waiting-room with a relative or friend. The three groups were chosen for being easy to implement in both a clinic and a domestic setting and for allowing to separate the effect of music from that of being comfortably seated. Randomization was achieved by sequentially allocating each woman to a group, using a random generator of integer digits (1—3 for music, 4—6 for reading, 7—9 for waiting-room). Four types of music compact discs (CD) were prerecorded for this study. Each CD contained approximately 30 min of music consisting of light vocals (tracks from Enya, Vangelis, Shaina Noll), light instrumental (Govi, Vangelis, Diane Arkenstone), classical music (Bach, Wolfgang Schulz, Debussy, Boccherini), or vocal jazz (Chet Baker, Ella Fitzgerald, Norah Jones, Julie London, Anne Ducros, Louis Armstrong). A 3 min demonstration CD, with excerpts of the four types of music, was also created and previously heard by the participants in the music group, so they could choose the type they considered more relaxing for them.

To assess anxiety, the Portuguese Spielberger's State and Trait Anxiety Inventory (STAI) (Spielberger et al., 1970; Silva and Campos, 1999; Ponciano et al., 2005; Daniel et al., 2006) self-rating questionnaires were given to be completed by the women before and after the 30 min relaxation period. The 20-item STAI has been widely used in examining feelings of apprehension, nervousness, and worry. The state STAI (S-STAI) measures how women feel "right now" and the trait

STAI (T-STAI) measures how they "usually" feel. The questionnaire scores range from 20 to 80, with higher scores indicating a higher level of anxiety. In Portugal, the STAI averages in a sample of normal student females ($n = 826$, 18–30 years old) are 40 for S-STAI (sd 10.7, range 20–78) and 42 for T-STAI (sd 9.0, range 24–72) (Daniel et al., 2006).

Before the 30 min period, a venous puncture was performed and a 20G Neo Delta Ven T intravenous catheter was applied to a peripheral arm vein and kept in place until the end of the intervention; 5 mL of maternal blood was collected before and after the 30 min period. The same variables were measured again in the end and the venous catheter was only then taken out as women entered another room for amniocenteses. Once drawn, maternal blood was immediately centrifuged for 10 min and the supernatant plasma was kept at $-80\,^{\circ}C$ for later assay. Total plasma cortisol was measured at the Department of Chemistry and Biochemistry of the Faculty of Sciences, University of Lisbon, by standard radio-immunoassay using Spectria cortisol RIA® coated tubes from Orion Diagnostica (Finland). The inter-assay coefficient of variation (CV) was 4.9% and the intra-assay CV was 4.4%.

We have compared cortisol levels and anxiety before and after the intervention. Numerical data were first checked for normality by examination of their histogram against the empirically expected Normal distribution and by inspection of P–P plots. Plasma cortisol and gestational age were log-transformed to approach a Normal distribution, which was achieved for cortisol but not for gestation (in practice, however, our results are identical with transformed and untransformed data). Time of day was discretized into intervals (08:30–9:00 h, 09:00–10:00 h, 10:00–11:00 h, 11:00–12:00 h, 12:00–13:00 h, 13:00–14:00 h, and 14:00–15:00 h). Maternal age, fetal sex, gestational age, parity, time of day, body mass index (BMI), and blood pressure were included as covariates in exploratory analyses as appropriate. Statistical analysis were conducted using SPSS version 17.0 (SPSS Inc., Chicago, IL, USA) and a $p$-value $<0.05$ was routinely considered significant. Analysis of variance (ANOVA) was used to compare group means, followed by post hoc pairwise test comparisons whenever the null hypothesis was rejected; analysis of covariance (ANCOVA) was used to compare group means controlling for the effect of one covariate, and multiple regression analysis was used to predict the value of an outcome variable from a set combination of predictors.

## 3. Results

Of the 157 patients recruited, 3 were excluded: one for impossibility of performing amniocentesis and two due to maternal pathology. The final sample consisted of 154 cases with complete demographic information, maternal cortisol data, and anxiety scores.

### 3.1. Measurements before relaxation

Women were on average 37.8 (sd 2.9) years old and their mean gestation age was 120.4 (sd 8.3) days. The fetus sex ratio, determined by karyotyping, was 51% boys and 49% girls. The average S-STAI score before the intervention was 43.2 (sd 11), a relatively high score (Teixeira et al., 1999), but the average trait anxiety level was lower (35.1, sd 9). Table 1 shows the main clinical characteristics of women before proceeding to relaxation. We found no significant differences between women attended in the morning (8:30–12:00 h) and in the afternoon (12:00–15:00 h) in regard to average state and trait. However, average plasma cortisol in the morning was significantly higher than in the afternoon (648 *versus* 547 nmol/L, $t$-test: $p = 0.004$).

State and trait anxiety were correlated ($r = 0.32$, $p < 0.001$) and younger women tended to have a higher S-STAI score ($r = -0.23$, $p = 0.005$). Gestational age also correlated with state, as women at lower gestation tended to be more anxious ($r = -0.18$, $p = 0.03$). Younger women at early gestation ages thus tended to be the most anxious ones. When age and gestation are trichotomised at the 33 and 66% percentiles, the average S-STAI of women at the lower percentiles of these variables is 47.7 (sd 12.2, $n = 15$) which is significantly different ($t$-test, $p = 0.03$) from the average S-STAI of 38.3 (sd 12.1, $n = 19$) at the higher percentiles. When pooled in multiple regression, trait, age, and gestation, explain 16.5% ($p = 0.004$) of the variance in the S-STAI anxiety score.

Maternal plasma cortisol, measured before relaxation, ranged from 239 to 1334 nmol/L. Cortisol tended to increase with gestation age ($r = 0.25$, $p = 0.002$) and to decrease with time of the day ($r = -0.30$, $p < 0.001$). Multiple regression analysis confirmed these associations as gestation and time of the day accounted for 13.7% of the variance in the Ln of cortisol ($p < 0.001$) after controlling for covariates like S-STAI (regression coefficient $b = 0.004$, $p = 0.09$), age ($b = 0.002$, $p = 0.8$), BMI ($b = -0.011$, $p = 0.11$), and T-STAI ($b = -0.002$, $p = 0.003$). The addition of the later covariates to a model already including gestation and time of day increases the variation explained to 16.7%, but this increment is not significant.

We have proceeded by exploring interactions between S-STAI and gestation, and S-STAI and time of the day. Women were first divided in two groups by the median gestation age of 118 days (~17 weeks). The partial correlation between

**Table 1** Characterization at arrival of women undergoing amniocentesis: mean values (standard deviation) [range] of maternal age, gestation, body mass index, fetus sex, State and Trait STAI anxiety scores, and plasma cortisol.

| | Total $n = 154$ | Morning $n = 88$ | Afternoon $n = 66$ |
|---|---|---|---|
| Maternal age (years) | 37.8 (2.9) [26.8–43.9] | | |
| Gestational age (days) | 120.4 (8.3) [110–165] | | |
| BMI (kg/m$^2$) | 24.9 (3.6) [18.4–37.1] | | |
| State-STAI before relaxation | 43.2 (11.5) [20–76] | 43.3 (11.4) [20–74] | 42.9 (11.7) [25–76] |
| Trait-STAI before relaxation | 35.1 (8.8) [21–75] | 34.4 (8.5) [21–75] | 35.9 (9.3) [21–68] |
| Maternal cortisol (nmol/L) | 604.8 (214.7) [239–1334] | 648.0 (222.4) [239–1334] | 547.2 (190.9) [270–1241] |

maternal cortisol and state anxiety (controlling for time of day) is not significant, neither in the ≤118 days group ($r = 0.05$, $p = 0.69$, $n = 78$) nor in the >118 group ($r = 0.13$, $p = 0.28$, $n = 76$). This result is in contrast with Sarkar et al. (2006), who reported a significant though weak correlation ($r = 0.178$, $p = 0.008$) between state anxiety and plasma cortisol below 17 weeks but not in more advanced gestational ages.

Women were then divided in two groups according to time of the day: a morning group (8:30—12:00 h) and an afternoon group (12:00—15:00 h). In the afternoon group the partial correlation between maternal cortisol and S-STAI was significant ($r = 0.25$, $p = 0.04$, $n = 66$), but not in the morning group ($r = -0.05$, $p = 0.62$, $n = 88$) (Fig. 1). Multiple regression analysis conducted with afternoon women alone, shows that S-STAI is the most important predictor of cortisol ($b = 0.007$, $p = 0.029$) accounting for 6.2% of its variability. Time of day follows in importance ($b = 0.171$, $p = 0.039$) and no other covariable (age, gestation, BMI, parity, trait anxiety), *de per si*, gives a statistically significant contribution although altogether they account for a significant 22.4% ($p = 0.032$) of variability in cortisol.

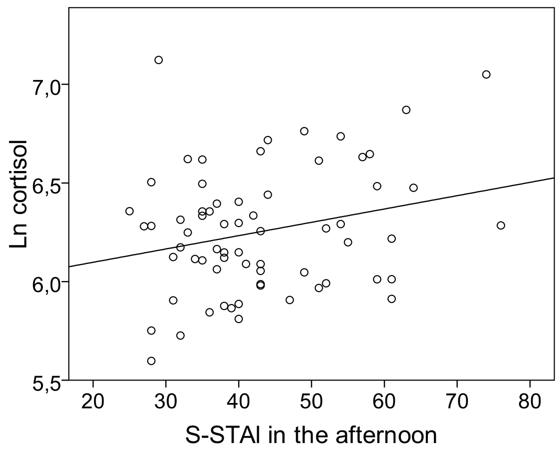


**Figure 1** Regression of Ln cortisol on S-STAI, measured in the afternoon, before relaxation. The regression line is $Y = 5.96 + 0.07X$ and accounts for 6.2% of variation in cortisol ($p = 0.04$).

## 3.2. Measurements after relaxation — S-STAI and T-STAI

Average anxiety scores decreased in all groups following the 30 min relaxation period (Table 2). The average global decrease in State was −6.0, being larger in the music group (−7.6) and smaller in the waiting-room (−4.5). Globally, the differences among groups are at the brink of statistical significance (ANOVA, $F_{2,151} = 2.90$, $p = 0.058$). We have proceeded by examining whether the initial S-STAI score somehow correlates with the subsequent change in S-STAI induced by relaxation. If this is the case, an uneven distribution of initial anxiety states among intervention groups might mask the effect of the different types of intervention. Partial correlation between initial S-STAI and the change in S-STAI is highly significant ($r = -0.32$, $p < 0.001$, controlling for age, time of day, T-STAI, and gestation): women who are more anxious before relaxation exhibit the greatest S-STAI decreases. Multiple regression confirms this finding. The type of relaxing intervention accounts for only 3.6% of variance in S-STAI change, but when the initial S-STAI is added to the model, the variance explained by the two variables jumps to 16% ($p < 0.001$).

In order to remove the effect of initial S-STAI when comparing the effect of the intervention groups in regard to decrease in S-STAI, we have conducted an analysis of covariance with initial S-STAI as covariate. The ANCOVA is highly significant in regard to the effect of type of relaxation intervention ($F_{2,150} = 7.3$, $p = 0.001$) and, as expected, in regard to the importance of the covariate ($F_{2,1} = 23.7$, $p < 0.001$). Multiple contrasts with a Bonferroni adjustment further indicate that the effect of music upon S-STAI change is significantly different from reading a magazine ($p = 0.014$) and from waiting in the waiting room ($p = 0.001$), but reading a magazine was not found different from waiting in the room ($p = 0.99$).

To illustrate the importance of the impact of the type of intervention upon S-STAI change, discriminated by the initial S-STAI score, the latter were trichotomised into low (<33rd percentile), medium (33rd—66th percentile) and high (>66th percentile) anxiety levels and the decrease in S-STAI was box-plotted by level and by intervention group (Fig. 2). Women with higher initial anxiety exhibited greater decreases within all groups and music was particularly effective at diminishing anxiety in these women.

Trait anxiety, expressing long-term tension and apprehension, and not expected to vary, diminished but much less than state, with an average global decrease of −1.5 (Table 2). The differences among groups concerning mean trait decreases were not significant (ANOVA, $F_{2,149} = 0.32$, $p = 0.73$) and removing the effect of initial T-STAI by analysis of covariance did not change this lack of significance either.

## 3.3. Measurements after relaxation — plasma cortisol

On average, plasma cortisol decreased −35.6 nmol/L (sd 154 nmol/L). A major correlate of change in cortisol was

**Table 2** Mean variation in S-STAI and T-STAI scores and in cortisol, by intervention group.

| Group | n | State STAI | | Trait STAI | | Cortisol (nmol/L) | |
|---|---|---|---|---|---|---|---|
| | | Mean D | St. Dev. | Mean D | St. Dev. | Mean D | St. Dev. |
| Music | 61 | −7.6 | 8.3 | −1.4 | 2.9 | −61.8 | 131.3 |
| Magazine | 46 | −5.5 | 6.4 | −1.3 | 2.7 | −49.7 | 104.7 |
| Wait-room | 47 | −4.5 | 5.7 | −1.8 | 3.9 | 12.2 | 156.8 |
| Total | 154 | −6.0 | 7.1 | −1.5 | 3.2 | −35.6 | 154 |

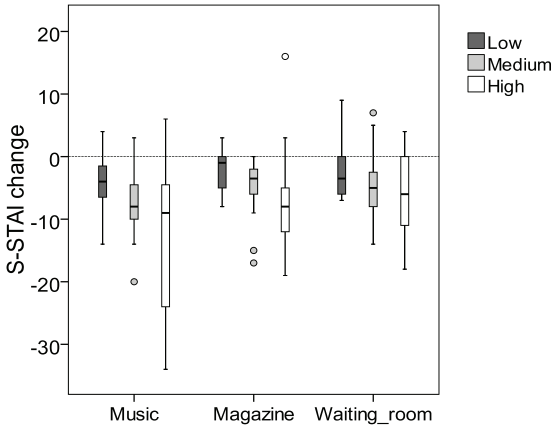


**Figure 2** Change in state STAI following relaxation, by type of intervention and by three levels of S-STAI before the intervention: low (<36), medium (36–41), and high levels (>45). Values below the dashed line are decreases in state anxiety.

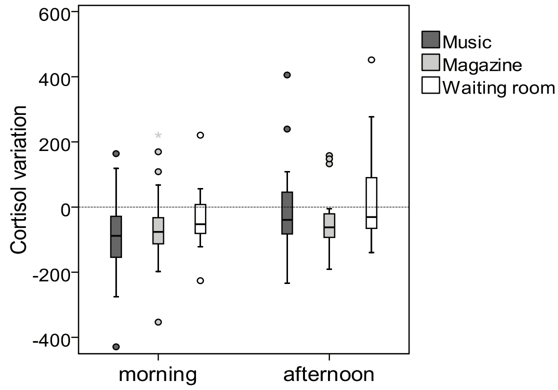


**Figure 3** Change in cortisol (nmol/L) following 30 min relaxation by type of intervention and time of the day. Values below the dashed line are decreases in cortisol.

time of the day ($r = 0.24$, $p = 0.003$), with major decreases tending to occur in the morning and minor changes taking place in the afternoon. On average cortisol changed −56.5 nmol/L (sd 134) in the morning and −7.6 nmol/L (sd 133) in the afternoon. Women with higher cortisol levels before intervention were the ones that tended to decrease the most during the 30 min relaxation (partial correlation, controlling for time of the day, $r = -0.23$, $p = 0.004$). The change in cortisol does not correlate with change in state anxiety ($r = 0.03$, $p = 0.70$) neither when pooled for all times of the day, nor when women are split into a morning and an afternoon group.

The larger decrease in cortisol occurred in the music group (−61.8 nmol/L) (Table 2). Women reading a magazine, on average, also decreased (−49.7 nmol/L) but those in the waiting-room increased their cortisol level (+12.2 nmol/L). These differences in cortisol response among intervention groups are globally significant (ANOVA $F_{2,151} = 4.5$, $p = 0.01$) and post hoc tests further indicate that the music and waiting-room groups are the most significantly different groups (Bonferroni $p = 0.01$, LSD $p = 0.01$). There is however no significant difference between the music and the magazine group (Bonferroni $p = 0.99$, LSD $p = 0.64$).

Multiple regression confirms these findings, indicating that initial level of cortisol, type of relaxing intervention, and time of the day, in this order, are the most important predictors of cortisol change. Altogether these variables account for a significant 14.6% ($p < 0.001$) of variation in cortisol change (Table 3). Addition of age, gestational age, BMI, and S-STAI change, increased the percentage of variation explained to 17.2%, but the 2.6% increment is not statistically significant ($p = 0.34$).

Furthermore, time of the day interacts with the way intervention groups affect cortisol change. When women are split into a morning and an afternoon group, the differences among types of intervention are on the borderline of significance in the morning (ANOVA $F_{2,85} = 3.2$, $p = 0.05$), but not in the afternoon (ANOVA $F_{2,63} = 1.5$, $p = 0.23$). Fig. 3 shows the combined effect of type of intervention and time of the day upon cortisol change. It becomes clear that the

**Table 3** Results of a 2 step hierarchical multiple regression on the change in cortisol (''cortisol after'' minus ''cortisol before'' relaxation). The $b$ are regression coefficients; $R^2$ is the variance explained at each level and $\Delta R^2$ is the increment due to the addition of covariates at each step; significant coefficients and variance explained are signaled by *<0.05, **<0.01, and ***<0.001.

| | | $b$ | Std error ($b$) | $p$ | $R^2$ | $\Delta R^2$ |
|---|---|---|---|---|---|---|
| Step 1 | Constant | 426.43 | 1.943 | 0.042 * | 0.146 | 0.146 *** |
| | Ln (initial cortisol) | −87.57 | 31.31 | 0.006 ** | | |
| | Music/magazine/waitroom | 31.28 | 12.32 | 0.012 * | | |
| | Time of day | 13.31 | 6.47 | 0.041 * | | |
| Step 2 | Constant | −383.04 | 791.54 | 0.629 | 0.172 | 0.026 NS |
| | Ln (initial cortisol) | −94.95 | 32.57 | 0.004 ** | | |
| | Music/magazine/waitroom | 29.18 | 12.79 | 0.024 * | | |
| | Time of day | 12.27 | 6.50 | 0.061 | | |
| | Age | −5.50 | 3.66 | 0.135 | | |
| | Ln (gestational age) | 207.04 | 166.63 | 0.216 | | |
| | BMI | 3.07 | 0.08 | 0.284 | | |
| | S-STAI change | −0.52 | 1.47 | 0.723 | | |

main decreases in cortisol took place in the morning, when music was used for relaxation.

## 4. Discussion

We have compared the impact of short-term interventions upon anxiety and cortisol, in pregnant women awaiting imminent amniocentesis, by means of an experimental setting that is easily portable to a clinic or a domestic environment. The association between stress, anxiety and hormonal secretion in human pregnancy is still poorly understood. Petraglia et al. (2001) and Obel et al. (2005) were among the first to study the association between self-reported psychological stress and cortisol during pregnancy. Sarkar et al. (2006) were the first to explore the cortisol response to the anxiety generated by imminent amniocentesis but they have used only morning samples and did not examine relaxation interventions. Teixeira et al. (2005) and Field et al. (2009) have examined the impact of relaxation by interventions, but these were not as prone to a quick and in expensive implementation as ours. In this study, we have recruited 154 women with an average age of 37.8 (sd 2.9) years old and mean gestation age of 120.4 (sd 8.3) days, about to undergo amniocentesis. Women were randomly assigned to three groups in order to examine the impact of non-pharmacological relaxation interventions aimed at curbing the psychological and cortisol response to an anxiogenic stressor during pregnancy.

### 4.1. Anxiety

The baseline state anxiety scores of women were relatively high (average 43.2, sd 11) confirming claims that imminent amniocentesis is perceived as an anxiogenic stressor (Ng et al., 2004; Sarkar et al., 2006). The average trait anxiety level was lower (35.1, sd 9) though, suggesting that these women were not inherently anxious. On average, younger women ($p = 0.005$) at early gestational ages ($p = 0.03$) were the most anxious ones. This result contrasts with the study of Sarkar et al. (2006), who found no association between gestation age and anxiety level, but is in agreement with the claim that, as pregnancy advances, the psychological response to stress becomes progressively attenuated. A possible explanation might be that elevated levels of hormones, such as cortisol and corticotropin-releasing hormone, would down-regulate the corticotropic system accounting for a dampened response of the HPA axis (Glynn et al., 2001, 2004).

The scores on both S-STAI and T-STAI decreased in all groups following the 30 min relaxation period. The average S-STAI decreased $-6.0$, with music being the most effective stimulus to decrease the self-perception of anxiety. With an average change of $-7.6$, music was better ($p = 0.001$, after controlling for the effect of the initial S-STAI level) than reading a magazine ($-5.5$) or just waiting ($-4.5$). It worked particularly well for the most anxious women, the ones benefiting the most in all types of intervention (Fig. 2). Listening to music may enhance relaxation by providing a distraction from a focus of awareness, such as an existing stress or physical pain source (Davis et al., 1999; Evans, 2002; Kwekkeboom, 2003). Distraction may combine with the physiological effects of music listening (for reviews see Schneck and Berger, 2006; Krout, 2007) being beneficial to the listener by enhancing the relaxation process.

### 4.2. Cortisol

Plasma cortisol in the morning was significantly higher than in the afternoon ($p = 0.004$).

Cortisol tended to decrease between 8:30 and 15 h ($p < 0.001$), in agreement with its well-known circadian rhythm (Weitzman et al., 1971; Veldhuis et al., 1989; Dickmeis, 2009), and tended to increase with gestational age ($p = 0.002$). From the coefficients of regression analysis, we found that, on average, 2.7 additional gestation days corresponded to more 3.4 nmol/L in the plasma cortisol, assuming everything else remains equal. Like Goedhart et al. (2010), we found that women with higher BMI tended to have lower plasma cortisol level but, unlike those authors, we found this association not statistically significant ($p = 0.11$).

The partial correlation between maternal cortisol and S-STAI was significant in the afternoon ($p = 0.04$), but not in the morning group ($p = 0.62$). S-STAI stands as the most important predictor of cortisol in the afternoon, and multiple regression showed that, on average, one additional unit of state anxiety in the afternoon corresponds to an increase of 1 nmol/L in plasma cortisol, assuming everything else remains constant. This finding corroborates the evening correlation between stressful events and salivary cortisol reported by Obel et al. (2005) for pregnant women. It appears that evening cortisol is more sensitive to stressful events, perhaps because morning levels are already so high that it would take more dramatic events to raise them further. The apparent absence of cortisol response to stress in the morning has also been reported for non-pregnant women (Powell et al., 2002).

The relaxing interventions promoted average decreases in cortisol, in addition to a generalized improvement in psychological well-being. The three most important variables accounting for cortisol change during the 30 min relaxation period were, in this order, the initial level of cortisol, the type of intervention, and the time of the day (Table 3). The first and third variables were to be expected from the cortisol circadian rhythm, but the comparison among the types of interventions used upon the response of cortisol is unique to this study. We have found significant differences among the relaxation groups, with music being the most effective intervention, especially when compared with the group that sat in the waiting room (Table 2). The greater average decrease in plasma cortisol took place in the morning for women in the music group. In the afternoon, the average change in cortisol was smaller, with no significant difference among groups. It is possible that a 30 min relaxation might not be long enough to reduce cortisol in the afternoon and/or that cortisol level in the afternoon is already too low for further decrease with relaxation.

### 4.3. Intervention

Complementary and alternative interventions are the most common choices for pregnant women attempting to avoid the side-effects associated with medication. The results of this study support the hypothesis that a relaxation period, as short as 30 min, is likely to have a positive effect on the psychological health of women subjected to stressful situations. These results also indicate that relaxing music, in particular, helps to decrease anxiety and plasma cortisol levels, being especially effective in the morning. Women who are prone to become too anxious are the ones that should benefit the most. Other authors have reported similar results with music listening, concerning the psychological well-being, but not cortisol on pregnant women (Browning, 2000; Sidorenko, 2000; Chang and Chen, 2005; Chang et al., 2008).

For practical applications it is important to consider the type of music used for listening, as music perceived to be soothing or relaxing to a person may not be so for another. Age, culture, and socioeconomic status affect the way people respond to music (Dunn, 2004; Krout, 2007). In this study the participants chose what they considered to be the most relaxing type of music for them, following previous studies indicating the efficacy of patient-selected music in perioperative periods (Kulkarni et al., 2002; Clark et al., 2006; Mitchell and MacDonald, 2006). In general, it has been shown that music chosen by a subject tends to have the most self beneficial effects on relaxation and stress reduction (Siedliecki and Good, 2006; Pelletier, 2004; Krout, 2007). The duration of the relaxing period is also important. A 30 min period was chosen, mainly because it would be easy to implement as part of routine procedures in clinical settings. We were also concerned on whether a too long exposure, in an unfamiliar environment, might have counterproductive effects upon women's anxiety. For domestic practice however, the duration of intervention, as well as music selection, might probably be left to the women's preference if kept within reasonable limits.

### 4.4. Study limitations

A number of limitations need to be acknowledged regarding this study. We ignore the full extent to which our findings can have domestic relevance. Women from very heterogeneous backgrounds were offered conditions for an half-hour relaxation which may not be reproducible at home. The sense of comfort and security offered in our clinical setting is relevant for relaxation to an extent that is unknown and might not have affected women in the three groups the same way. The practical implementation of music relaxation in the clinic should not raise any major difficulty. We suggest it might be conducted while waiting in a cozy and convenient room, located nearby where amniocentesis or other invasive technique will take place. Nevertheless, our results may also have limitations when drawing conclusions for clinical applications as women in the music and magazine groups relaxed in isolation from other patients that might eventually cause disturbance or distraction. Finally, we were limited to the population of women that did not fall into our exclusion criteria. In particular, we do not claim that they apply equally well to women with underlying medical conditions and/or under medication, who might for some reason stray from average responses to relaxing interventions.

## 5. Conclusion

This study suggests that pregnant women are likely to benefit from routine practice of a 30 min relaxation period when faced with the imminence of a stressful event. The benefit would be both psychological and physiological and would be greater in the morning. Women prone to become too anxious, typically younger and at earlier gestational ages, are the ones that should benefit the most.

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## Conflict of interest

None declared.

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