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# Assessment of the oxidative status in mother-child couples from Seville (Spain): A prospective cohort study

Bouchra Dahiri [a], María G. Hinojosa [b,*], Pilar Carbonero-Aguilar [a], Lucas Cerrillos [d], Rosa Ostos [c], Juan Bautista [e], Isabel Moreno [a]

[a] Area of Toxicology, Department of Nutrition and Bromatology, Toxicology and Legal Medicine, Faculty of Pharmacy, University of Sevilla, 41012, Sevilla, Spain
[b] Department of Biochemistry and Biophysics, Stockholm University, Institutionen för biokemi och biofysik, 106 91, Stockholm, Sweden
[c] Department of Genetics, Reproduction and Fetal Medicine, Hospital Universitario Virgen del Rocío, Avda. Manuel Siurot, 41013, Sevilla, Spain
[d] Department of Gynaecology and Obstretrics, Hospital Universitario Virgen de Valme, Ctra. de Cádiz, 41014, Sevilla, Spain
[e] Department of Biochemistry and Molecular Biology, Faculty of Pharmacy, University of Sevilla, 41012, Sevilla, Spain

A R T I C L E I N F O

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A B S T R A C T

Pregnancy requires a high demand of energy, which leads to an increase of oxidative stress. The aim of this study was to assess the oxidative status in 200 couples of pregnant women-newborns at the time of delivery, for the first time, who gave birth in two University Hospitals from the province of Seville. Recruited women filled an epidemiological questionnaire with their demographic characteristics and dietary habits during pregnancy. At the time of delivery, both maternal and cord blood samples were collected. Protein oxidation, superoxide dismutase, and catalase levels were measured to assess the oxidative status of these women, together with the levels of vitamins D, B12, Zn, Se, and Cu. Our results showed a tendency for all biomarkers measured to be higher in cord blood than in maternal blood. For the correlations established between the OS markers and sociodemographic characteristics, only significant differences for carbonyl groups values were found on both maternal and cord blood, relating these higher values to the use of insecticides in the women's homes. For newborns, only a significant correlation was detected between antioxidant enzymes and the newborn's weight, specifically for superoxide dismutase activity. Additionally, the higher values obtained in cord blood might suggest metabolization, while a higher production of ROS and antioxidant enzymes might be required to maintain the balance. Measured levels for Se were similar in both maternal and cord blood, unlike Cu and Zn, where higher levels were found for maternal blood than cord blood, indicating a correlation between maternal Se values and SOD as OS biomarker. Furthermore, vitamin D levels were around the optimum values established, finding a relationship between vitamin D and new-born's height, unlike for vitamin B12 values, where a correlation with maternal food consumption characteristics was established. Overall values were inside normal ranges and consistent for our population.

## 1. Introduction

Oxidative stress (OS) is "the imbalance between production of free radicals and reactive metabolites, and their elimination by (…) antioxidative systems" [1]. In this sense, reactive oxygen species (ROS) are mainly the result of metabolic processes in cellular organelles, and in case of immune activation, inflammatory processes, infections, cancer, ischaemia, or even when exercising at high intensity [2]. In general, low concentrations of ROS are required in cellular signalling [3], and immune responses [4,5] (Fig. 1). If this balance is disturbed, ROS may accumulate and become detrimental, causing many pathologies, such as neurodegenerative disorders (Alzheimer's and Parkinson's diseases among others [6–11] [6–11] [6–11]), cardiovascular diseases (such as atherosclerosis, or Diabetes Mellitus) [12–14], or even cancer [15].

During pregnancy, a high demand of energy is produced [16]. A healthy placenta already causes an increase of OS levels, but these levels need to be accompanied by an increase of antioxidant protection with the activity of enzymes such as superoxide dismutase (SOD) or catalase (CAT), to compensate ROS production and lead to a healthy pregnancy [16,17] (Fig. 2). Levels of ROS play a regulatory role on the pathways of

* Corresponding author.
*E-mail address:* maria.hinojosa@dbb.su.se (M.G. Hinojosa).

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folliculogenesis, oocyte maturation, and corpus luteum and uterine function, and they are also related to embryogenesis, embryo implantation, and fetoplacental development [18]. In this regard, different pregnancy related-diseases can occur, including gestational diabetes, spontaneous abortion, pregnancy loss, and preeclampsia, among others [19]. During pregnancy loss or spontaneous abortion, lipid peroxidation (LPO)-products reach higher levels immediately before these episodes [20].

Considering all of this, and that the most relevant free radicals have a short half-life, most OS studies use indirect measures such as the activity of SOD and CAT [21–23] [21–23] [21–23], or the products resulting from oxidative damage [24]. Furthermore, altered activity of one antioxidant enzyme without compensatory changes in others may lead to LPO [24]. The same happens with other indirect markers, such as protein carbonylation as a hallmark of protein oxidation [25], and DNA oxidation as a marker of DNA damage [26].

Besides these biomarkers, there are some other factors able to play a role in this redox balance, being this the case of some metals. In this regard, redox active metals participate in the transferring of electrons between metals and substrates, being crucial in the maintenance of redox homeostasis [27,28]. Thus, disruption of metal homeostasis can lead to the formation of free radicals that may participate in the modification of DNA bases, and increase LPO levels, among others [27]. Among all of them copper (Cu), selenium (Se) and zinc (Zn) are of special interest.

Copper is a metal that occurs naturally in soil and water, while anthropogenic activities such as mining, industrial discharges, and copper-based pesticides can be sources of Cu-water contamination [29,30]. Besides participating in biological systems as electron transport, Cu is a cofactor of many enzymes involved in redox reactions, such as cytochrome C oxidase, ascorbate oxidase, or SOD [27,28]. This metal can induce OS by the formation of ROS via Fenton reaction, which could lead to DNA strand breaks and oxidation of bases, or through the binding to thiol-containing molecules such as glutathione, which would decrease glutathione levels by activation of MAPK signalling cascades [27,31]. On the other hand, Cu plays a protective role against oxidative damage through ceruloplasmin and metallothioneins [29].

Concerning selenium, it is a trace element that can be obtained from grains, cereals, meat, or supplementation [32]. This metal plays an antioxidant role, as is an essential cofactor for GPx, selenoprotein P, and thioredoxin reductase, which are involved in scavenging free radicals and maintaining the redox balance [33,34]. Furthermore, it has been observed an increase in plasma GSH and a significant reduction in plasma MDA levels after the intake of selenium supplements in pregnant women with gestational diabetes for instance Ref. [34]. In addition, these supplements have also demonstrated to cause some beneficial effects for some diseases caused by OS, such as cardiovascular and inflammatory diseases or even cancer [34].

Zinc is a ubiquitous trace element found in animals and plants and plays an important role in the metabolism of proteins, lipids, and carbohydrates [27]. In general, this metal has an essential role in immunodevelopment, and its deficiency may cause growth retardation and hypogonadism in humans [27]. In general, this metal can exert an antioxidant function through the protection of sulfhydryl groups of proteins against free radical attack, and through the reduction of free radical formation by antagonism of metals such as Fe or Cu, which would lead to a Fenton reaction otherwise [18,27]. In addition, Zn inhibits NADPH oxidase and reduces inflammatory cytokine production, while Zn-deficiency has been linked to high levels of OS by increased lipid, protein, and DNA oxidation [27].

However, metals are not the only molecules able to interfere with the OS status in the human body, as the presence of some vitamins is also a factor to consider. In general, vitamin D deficiency is considered a global public health issue attributed to decreased sun exposure and insufficient intake of vitamin D rich food, and this deficiency has been associated to the development of infectious, autoimmune, and cardiometabolic diseases [35–37]. In this sense, vitamin D supports cellular redox reactions by maintaining normal mitochondrial functions, avoiding aberrant cell proliferation, cell death neurodegeneration, and accelerated aging [37]. Furthermore, some studies have pointed out the antioxidant properties of vitamin D, as reviewed in Filgueiras et al. (2020) [36], finding association between vitamin D and OS biomarkers such as SOD, GPx, and upregulation of cellular GSH, causing a decrease of ROS levels [38].

Lastly, B-group vitamins are essential for optimal body and brain function, and their shortage can lead to high levels of neural inflammation and oxidative stress [39]. In this regard, vitamin B12 deficiency has been linked to several syndromes related to megaloblastic anaemia and disorders of the nervous system, which may be due to the accumulation of homocysteine, which could lead to reduced myelination and brain atrophy in adults [39–41] [39–41] [39–41].

Thus, the purpose of this study is to assess the oxidative status of a cohort of 200 pregnant women-child couples living in different locations in Seville province (Spain), being the first human biomonitoring study carried out in this population, by taking into account their habits and characteristics. For this, the measurement of different oxidative stress parameters including protein oxidation, SOD and CAT, metals involved in OS status (Cu, Se and Zn) and vitamins such as vitamin D and B12 was carried out. Secondary goals were to evaluate sociodemographic characteristics, day to day, and nutrition habits, to try to establish a relationship between the oxidative stress parameters measured and the sociodemographic characteristics, lifestyle, and dietary habits.

## 2. Methods

### 2.1. Study population

The present study is based on ongoing prospective birth cohorts in the south of Spain. Participants in this study were pregnant women who

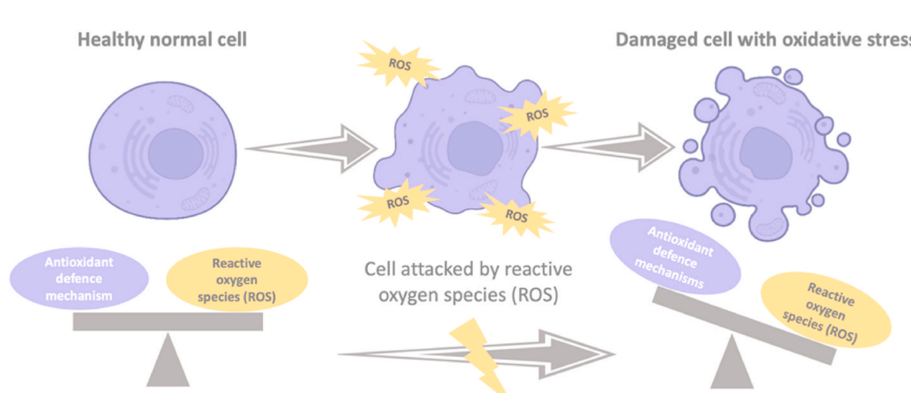


**Fig. 1.** Healthy and damaged status of cells created by the imbalance between ROS production and antioxidant defence mechanisms. Created using powerpoint.

gave birth during July 2020–2022 in two hospitals located in the city of Seville (Spain): Virgen del Rocío University Hospital (VRUH) and Virgen de Valme University Hospital (VVUH). Participation was offered to all women that went into delivery at the time and who meet inclusion criteria, enrolling those who accepted and signed informed consent. The cohorts were established considering the residence area of the participants (Seville city (SC), villages from Southern Seville area (SSA), and villages from Aljarafe area (AA)) (Fig. 3). The Women's Hospital, located in the VRUH health complex, serves a population of 554,981 inhabitants (mostly neighbourhoods from the southern district of SC, and villages from the AA) and performs more than 6,000 assisted deliveries a year. VVUH covers a population of 350,563 inhabitants and has a mean average of 3,100 deliveries per year, covering the rest of neighbourhoods from the southern district of SC and the villages from the SSA.

The inclusion criteria were the following: I. Age between 18 and 40 years; II. Residence in the reference area for, at least, 5 years; III. Single pregnancy; IV. Have not undergone a process of assisted reproduction; V. No communication problems; VI. Giving birth in any of the two reference hospitals.

The exclusion criteria were the following: I. Diabetes or hypertension; II. Other chronic and/or contagious diseases; III. Multiple birth; IV. Tested positive for COVID19; V. Additional exclusion criteria for the new-born: free from impaired psychomotor development, chromosome disorders, central nervous system malformations, symptoms of neonatal hypoglycemia, prolonged mechanical ventilation, or psiconeurosensory risk (derived from early birth, intrauterine congenital infections, and bilirubin levels >25 mg/dL).

All participants signed an informed consent. Furthermore, an epidemiological questionnaire was given through a QR code, which contained questions about their demographic characteristics (age, place of residence and birth, etc.), habits (smoking and alcohol drinking), and dietary habits (type of diet, frequency of consumption of rice, fish, canned foods, etc.) during pregnancy.

All enrolled participants have been split in by groups depending on their different living areas, based on the answers from the epidemiological questionnaire. Women living in SC (population of 688,711 inhabitants in 2020, being the fourth most populated city in Spain): big cities present more air pollution, primarily due to urban traffic. On the other hand, the pregnant women living in the villages of the SSA and AA areas are less exposed to this air pollution. The SSA area is integrated by 19 towns, with a population of 88,113 inhabitants, being only the 4.7 % of the whole population of the province. It is a completely rural environment located around a mountain chain, with mostly smaller villages. The AA is bigger and integrated by 30 towns, only 7 km from the Atlantic Ocean and separated from Seville city by the Guadalquivir River. The AA is a more populated metropolitan area with 390,772 habitants.

The study was approved by Coordinating Committee for the Ethics of Biomedical Research of Andalusia in June 2020 (1479-N-20) and written informed consent was obtained from the participants.

#### 2.1.1. Collection and processing of human peripheral and cord blood samples

From July 2020–2022, 200 pregnant women were recruited to participate in this study, obtaining blood samples from both the women and the new-born. It is important to note that baby blood samples were represented by cord blood samples, to avoid disturbances and exposure of the babies to additional health risks. Three tubes of both peripheral maternal and cord blood samples were taken. Two of the samples were stored in EDTA vacuum collection tubes (Vacutainer®) and the other was stored in a serum gel separation tube (Vacutainer®). The collected samples were carefully transported from the hospitals to the laboratory in a refrigerated box and processed immediately upon arrival to avoid contamination and hemolization.

One of the EDTA tubes was used to isolate the plasma. For this purpose, the method established by the Spanish National Biobank Network was used. Briefly, the samples were centrifuged twice and then separated in three different phases: plasma, leucocytes, and erythrocytes. Then, the upper phase corresponding to plasma was separated and transferred into a sterile tube, and was submitted to a second centrifugation, to eliminate any residual platelets or leucocytes. Finally, the plasma was transferred to several 1.5 mL tubes and stored at −80 °C until the measurements were done.

The gel separation tube was used to isolate serum. Following the standard procedure for this type of Vacutainer tube, the samples were centrifuged twice, obtaining three different phases: serum, separator gel, and blood cells at the bottom. The upper serum phase was transferred in to 1.5 mL tubes and stored at −80 °C until the measurements were done. These serum samples were only used when there was no plasma available for the measurements and serum samples were allowed.

#### 2.1.2. Evaluation of protein oxidation by quantification of carbonyl groups

Protein oxidation was studied by quantification of carbonyl groups, following the method proposed by Levine et al. [42] which is based on the reaction between 2,4-Dinitrophenylhydrazine (DNPH) and the carbonyl groups that would be present in the sample in case of protein oxidation. As a result of this reaction, a coloured product called 2,4-dinitrophenylhydrazone is produced. Values were expressed in moles of

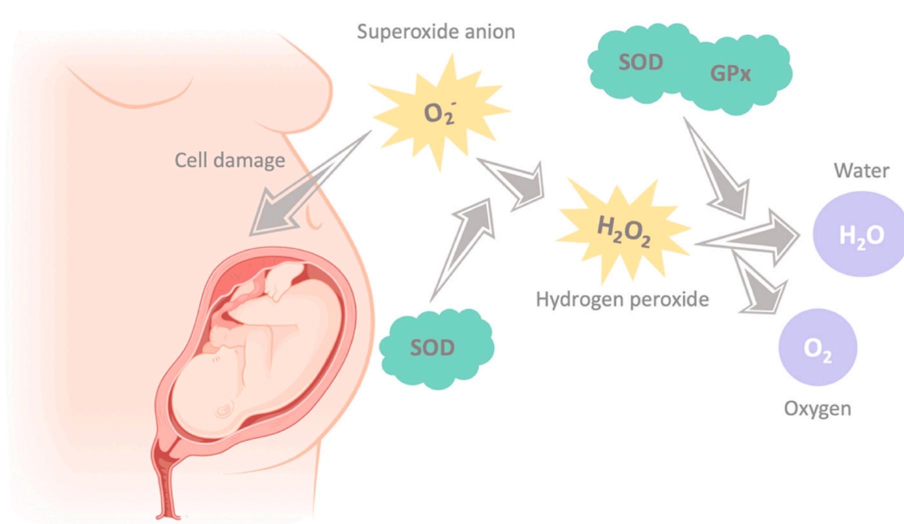


**Fig. 2.** SOD and CAT activity against the ROS production inside the placenta. Created using powerpoint.

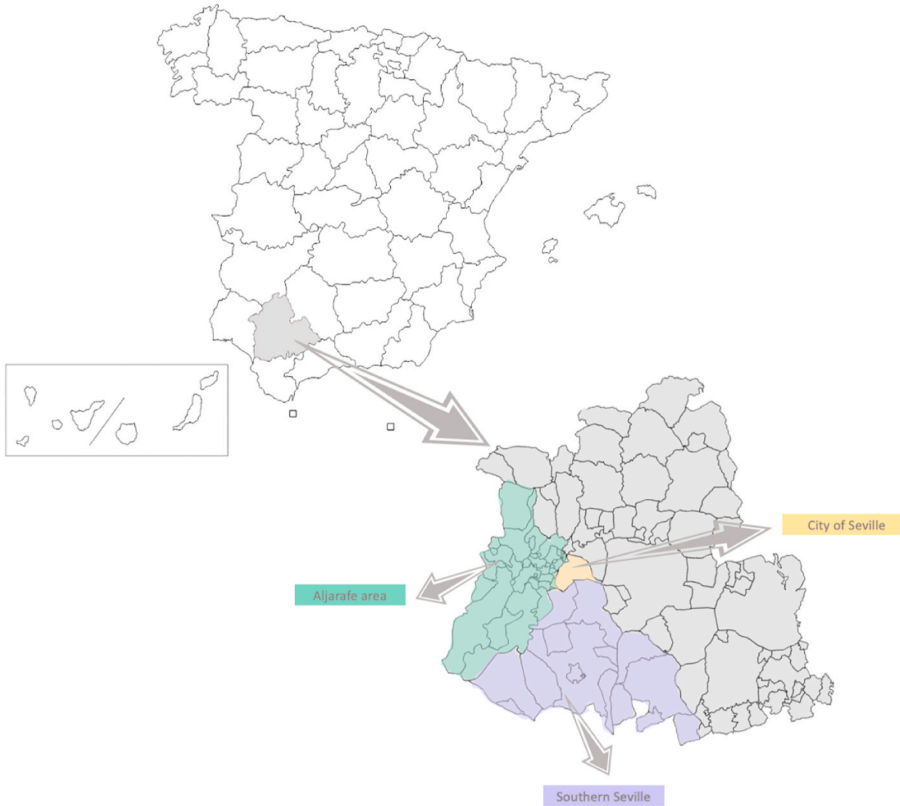


**Fig. 3.** Area of residence of participants. Created using powerpoint.

carbonyl groups per mg of protein.

*2.1.3. Evaluation of superoxide dismutase (SOD) activity*

For this purpose, a Superoxide Dismutase Assay Kit (Cat. 706002, Cayman®) was used and the assay was performed following the manufacturer's instructions. The absorbance was read at 440–460 nm using a plate reader and expressed at U per mL of serum.

*2.1.4. Evaluation of catalase (CAT) activity*

Catalase activity was evaluated using a Catalase Assay Kit (Cat.707002, Cayman®), following the instructions provided by the manufacturer. This assay is based on the peroxidation reaction of the catalase enzyme with methanol in the presence of $H_2O_2$ at an optimal concentration. This produces formaldehyde, which is measured colorimetrically using 4-amino-3-hydrazino-5-mercapto-1,2,4-triazole (Purpald) as a chromogen that produces a purple colour. The assay was performed in a 96-well plate for analysis and absorbance was read at 540 nm using a plate reader. The units of measurement for CAT values are nmol/min per mL of sample.

*2.1.5. Determination of Cu, Se and Zn*

The concentrations of Cu, Se and Zn were assessed in maternal and cord blood samples collected during delivery. An Anton Paar Multiwave 3000 system was used to digest blood. Detection limits were calculated through the concentration of an element, giving a signal equal three times the standard deviation of a series of ten measurements of the blank solution at the element peak. The average detection limits in blood were 0.51 ng/mL for Cu, 0.19 ng/mL for Se and 5.46 ng/mL for Zn.

Samples were predigested in the presence of $HNO_3$ first and incubated with $H_2O_2$ added. The digestion program consisted of initial power ramp from 0 to 800 W of 10 min, followed by a continuous power stage of 800 W for 20 min, and a final cooling stage of 15 min, with no power applied. Water and $HNO_3$ is added to the samples to perform the digestion. All the measured samples were prepared in triplicate.

Inductively coupled plasma mass spectrometric measurements were performed with an Agilent 7500c ICP-MS (Agilent Technologies, Tokyo, Japan) equipped with an Integrated Autosampler and an Octupole Reaction System (ORS).

*2.1.6. Determination of vitamin D and B12*

Vitamin D (in the form of 25-OH-D) and B12 were measured in maternal blood samples obtained at the time of delivery. The samples were analyzed through an IDS-iSYS multi-discipline automated immunoassay system in the different hospitals in which delivery took place.

*2.1.7. Statistical analysis*

Results were classified in qualitative and quantitative variables; thus, qualitative variables were expressed in percentage and frequency terms and quantitative ones were expressed as means with standard deviation, or median, and sometimes 25 and 75 percentiles or minimum and maximum were given.

A Shapiro-Wilk test was run to determine whether the data followed a normal distribution model. Depending on the results of that test, Pearson or Spearman's correlation coefficients were used to determine the correlation between maternal and cord blood results. The correlation between these results and the lifestyle and habits of the pregnant woman was also studied trough Pearson or Spearman's or Student's t-test or Mann-Whitney.

Data were listed on several Excel 16.55 accounting sheets and statistical analysis was performed using IBM SPSS Statistics 26. A significance level of $p = 0.05$ was established to accept or reject the hypothesis.

## 3. Results

### 3.1. Study population

General characteristics of the 200 included pregnant women and infants are presented in Tables 1 and 2. Taking into account the hospitals

**Table 1**
Mothers' characteristics.

| | | City of Seville (n=84) | Aljarafe area (n=30) | Southern Seville area (n=86) |
|---|---|---|---|---|
| Age (years) | | 33.67 (± 3.77) | 31.67 (± 5.87) | 33.18 (± 4.08) |
| Education | **Primary education** | 6.00 % | 10.00 % | 11.80 % |
| | **Secondary education** | 24.10 % | 23.30 % | 27.10 % |
| | **Higher education** | 69.90 % | 66.70 % | 61.20 % |
| Employment | **Unemployed** | 19.00 % | 20.00 % | 26.40 % |
| | **Employed** | 81.00 % | 80.00 % | 75.60 % |
| | **Working exposition** | 14.50 % | 33.30 % | 21.20 % |
| Smoking habits before pregnancy | **Smokers** | 16.70 % | 16.70 % | 14.00 % |
| | **Non-smokers** | 83.30 % | 83.30 % | 86.00 % |
| Smoking habits during pregnancy | **Smokers** | 7.10 % | 20.00 % | 11.60 % |
| | **Non-smokers** | 92.90 % | 80.00 % | 88.40 % |
| Dietary habits | **Mediterranean** | 97.60 % | 100.00 % | 100.00 % |
| | **Vegetarian** | 2.40 % | 0.00 % | 0.00 % |
| | **Vegan** | 0.00 % | 0.00 % | 0.00 % |
| | **Ecological fruit and vegetables** | 25.00 % | 16.70 % | 23.30 % |
| | **Average fruit pieces (times a day)** | 2.12 (± 1.16) | 2.00 (± 0.97) | 2.02 (± 1.12) |
| | **Average vegetable pieces (times a day)** | 1.65 (± 0.73) | 1.89 (± 1.05) | 1.44 (± 0.75) |
| | **Average legume servings (times a week)** | 2.12 (± 1.22) | 1.88 (± 0.86) | 2.29 (± 1.18) |
| | **Average rice servings (times a week)** | 1.56 (± 0.86) | 1.80 (± 1.19) | 1.87 (± 0.85) |
| | **Average sea-food servings (times a week)** | 1.86 (± 0.97) | 1.70 (± 0.88) | 1.63 (± 0.87) |
| | **Most consumed type of seafood** | White fish | White fish | Blue fish |
| | **Average canned sea-food servings (times a week)** | 1.23 (± 0.93) | 1.29 (± 1.12) | 1.55 (± 1.22) |
| | **Average canned non-fish servings (times a week)** | 1.09 (± 1.02) | 0.96 (± 1.43) | 1.49 (± 1.91) |
| Insecticides and pesticides | **Pet insect repellents** | 32.80 % | 58.30 % | 45.10 % |
| | **Insecticide use** | 77.40 % | 91.70 % | 76.50 % |
| | **Most common type of insecticide** | Plug-in | Plug-in | Spray |
| | **Chemical insecticides** | 81.80 % | 93.80 % | 79.30 % |
| | **Natural-made insecticides** | 18.20 % | 6.30 % | 20.70 % |
| | **Insecticides in baby's room** | 25.00 % | 35.00 % | 13.70 % |
| | **Garden** | 73.80 % | 80.00 % | 59.30 % |
| | **Pesticides in garden** | 11.30 % | 16.70 % | 13.70 % |

where participants gave birth, 66% of VRUH women lived in SC, 29.1% in AA, and 4.9% in SSA. In contrast, only 16.5% of VVUH women lived in SC, coming most of the participants from the SSA (83.5%).

The mean age of the women (N = 200), at the time of delivery, was 33.67 (± 3.77) years (range 24–40 years) for the participants of SC, 31.67 (± 5.87) years (range 22–41 years) for the participants of AA, and 33.18 (± 4.08) years (range 22–40 years) for the participants of SSA.

Concerning employment, 81, 80 and 15.6% of the women from SC, AA, and SSA, respectively, were employed, and 14.5% of them were exposed to heavy metals or pesticides in their workplace. A higher percentage, 33.3% of the AA women, were exposed to those contaminants in their workplace, while only 21.2% of the employed women from SSA declared being exposed to them.

In terms of lifestyle and habits, 16.7% of the women from SC and AA were smokers before pregnancy, with an average of 4.5–5 cigarettes per day; however, only 7.1% of women from SC declared smoking during pregnancy, with an average of 2 cigarettes per day. Similarly, regarding AA-women, 20% of them stated smoking during pregnancy, with an average of 4 cigarettes per day. About SSA, 14% of women declared smoking before pregnancy, with an average of 8 cigarettes per day, and 11.6% were smokers during pregnancy, reducing the average in 2 cigarettes per day.

**Table 2**
New-borns' characteristics.

| | | City of Seville (n=84) | Aljarafe area (n=30) | Southern Seville area (n=86) |
|---|---|---|---|---|
| Delivery type | **Eutocic** | 59.00 % | 57.10 % | 52.90 % |
| | **Vacuum-assisted** | 15.40 % | 10.70 % | 21.20 % |
| | **Forceps** | 3.80 % | 3.60 % | 4.70 % |
| | **Spatula-assisted** | 0.00 % | 3.60 % | 1.20 % |
| | **Cesarean section** | 21.80 % | 25.00 % | 20.00 % |
| Newborn weight (g) | | 3440.13 (± 459.11) | 3457.04 (± 477.43) | 3388.56 (± 425.91) |
| Newborn height (cm) | | 49.93 (± 1.98) | 50.25 (± 2.62) | 50.42 (± 1.86) |
| Head circumference (cm) | | 35.00 (± 1.46) | 35.02 (± 1.66) | 39.92 (± 1.40) |
| Cord pH | | 7.28 (± 0.75) | 7.29 (± 0.61) | 7.29 (± 0.68) |
| Sex | **Female** | 57.10 % | 55.60 % | 37.60 % |
| | **Male** | 42.90 % | 44.40 % | 62.40 % |

Regarding dietary habits, 97.6% of SC women stated following a Mediterranean diet and only 2.4% followed a vegetarian one. All the participants from the other two cohorts stated following a Mediterranean diet. Consumption of ecological fruit and vegetables was around 17–25% in the three cohorts.

Regarding pesticides, 32.80% of SC-women declared having pets and using insect repellent spots-on or flea collars. In addition, 77.40% stated using any type of insecticide, plug-in being the most common type (52.00%). Among them, 81.80% were common insecticides made from chemically toxic components, of which 25.00% were used in the baby's room. Lastly, 73.80% declared having a garden at home, and 11.30% of them stated using pesticides to prevent plagues in it. About AA-women, 58.30% declared having pets and using the same type of repellents mentioned above. 91.70% stated using any type of insecticide, plug-in being the most common type of it (61.90%). In addition, 93.80% of those who used plug-in insecticides stated using chemical-based insecticides and only 6.30% of them were used in the baby's room. Lastly, 80% declared having a garden at home, and 16.70% of them used pesticides. Concerning SSA women, 45.10% declared having pets and using the same repellents abovementioned. Insecticides were used by 76.50% of the women, being spray the most common type of them (54.80%). Among them, 79.30% were conventional chemical insecticides, 13.70% of them were used in the baby's room. Finally, 59.30% declared having a garden, 13.70% of them used pesticides.

Furthermore, Table 2 shows the characteristics of the new-borns. The eutocic or natural birth was the most common delivery type in all three cohorts (59.00% for SC, 57.10% for AA, and 52.90% for SSA). The average weight of SC new-borns was 3440.13 g (± 459.11), and the average length was 49.93 cm (± 1.98). Concerning new-borns from AA, their average weight and length were 3457.04 g (± 477.43) and 50.25 cm (± 2.62), respectively. Quite similar average weight (3388.56 g ± 425.91) and length (50.42 ± 1.86) were obtained for new-borns from SSA. The head circumference, and the cord pH values were also similar in the three areas. About the sex, 57.10% of SC new-borns were girls and 42.90% were boys. The same ratio was reported in AA, with a 55.60% of girls and 44.40% of boys. However, in SSA, the ratio was 37.60% girls, and 62.40% boys.

### 3.1.1. Evaluation of OS biomarkers, enzymatic activities, metals, and vitamins

A common scenario can be observed, which is that for the oxidative stress levels, the maternal results tend to be lower than the ones found in cord blood.

Regarding protein oxidation, measured by the concentration of carbonyl groups, the median values found in the cohort from SC (Fig. 4a) were $7.73x10^{-5}$ and $7.83x10^{-5}$ mol/mg protein, for maternal and cord blood, respectively. In pregnant women from AA, the median values obtained for maternal and cord blood were $6.55x10^{-5}$ and $9.42x10^{-5}$ mol/mg protein, respectively. The median values obtained in maternal and cord blood for SSA cohort were $5.90x10^{-5}$ and $8.27x10^{-5}$ mol/mg protein, respectively. We could not find significant differences between any of these groups in neither maternal samples nor cord blood samples from the different areas.

For catalase activity levels (Fig. 4b), the median values found in in SC cohort for both samples were visually significantly different (72.84 and 144.41 nmol/min/mL for, respectively) but no statistically significant differences were found between both type of samples and the different zones of residences. For AA, the median values found were 66.56 and 196.22 nmol/min/mL, respectively. Also, in SSA, significant differences were observed between maternal and cord blood, though level in maternal blood were the highest in the three cohorts and difference with blood cord levels was shorter (91.87 and 158.03 nmol/min/mL respectively).

Regarding SOD activity (Fig. 4c), similar median values in maternal blood in the three cohorts (1.87–2.38 U/mL) can be observed, being all of them lower than those detected in cord blood. However, levels detected in cord blood were significantly different ($p = 0.001$) between the cohort from SC (3.74 U/mL) and the other two from AA and SSA (5.34 and 5.10 U/mL, respectively).

As for vitamin D levels measured in maternal samples in the three cohorts, they all followed a similar distribution (Fig. 5a). For vitamin D levels, the highest values were the ones found in the SSA (46.40 ng/L), being significantly different ($p = 0.001$) to the SC (22.50 ng/L) and the AA (18.80 ng/L). For B12 levels (Fig. 5b), no significant differences were found for the three cohorts, being the highest median the one for SC (261.50 pg/mL), followed by the SSA (249.50 pg/mL) and the AA last (219.00 pg/mL).

Regarding Se levels, similar median values in maternal and cord blood in the three cohorts can be observed. For SC levels detected were 119.78 μg/L for maternal blood and 114.48 μg/L for cord blood. For AA 118.93 μg/L were the levels found for maternal blood against the 127.20 μg/L for cord blood. The SS levels were the lowest found for maternal blood (116.60 μg/L) and the intermediate values for cord blood (113.42 μg/L). The median values of Cu and Zn found in maternal blood were almost three times fold higher than the ones found in cord blood for every cohort. For SC, Cu levels were 1643.00 μg/L and Zn levels were 5077.40 μg/L for maternal blood and 647.66 μg/L and 2087.67 μg/L for Cu and Zn, respectively for cord blood. Maternal blood Cu levels in the AA were 1462.80 μg/L against the 614.80 μg/L for cord blood. For this area, maternal Zn levels were 5088.00 μg/L and cord blood levels were 2151.80 μg/L. As for the SS levels, maternal Cu median was 1633.46 μg/L and 657.20 μg/L for cord blood. Zn levels in this area were 5300.00 μg/L for maternal blood and 2268.40 μg/L for cord blood (see Table 3).

### 3.1.2. Study of correlation between maternal and cord blood OS markers, enzymatic activities and metals

Correlation statistics of protein oxidation and enzymatic activities of SOD and CAT found in maternal and cord blood are presented in Tables 4–6.

The Spearman's correlations observed were quite different between the three established cohorts. The strongest correlation between OS markers was observed in maternal and cord blood in CAT activity for the AA cohort. The correlations were positive and moderate, being $\boldsymbol{\rho} = 0.56$ ($p < 0.05$, establishing a range for $\boldsymbol{\rho}$ between 0.46 and 0.65 with a 95% confidence). For the SC, the strongest correlation, being positive and moderate, was also the one found for the carbonyl groups ($\rho = 0.50$; $p < 0.0001$, establishing a range for ρ between 0.38 and 0.60 with a 95% confidence). For the SSA, the strongest correlation was also the one found for the carbonyl groups levels, positive and a little lower but also

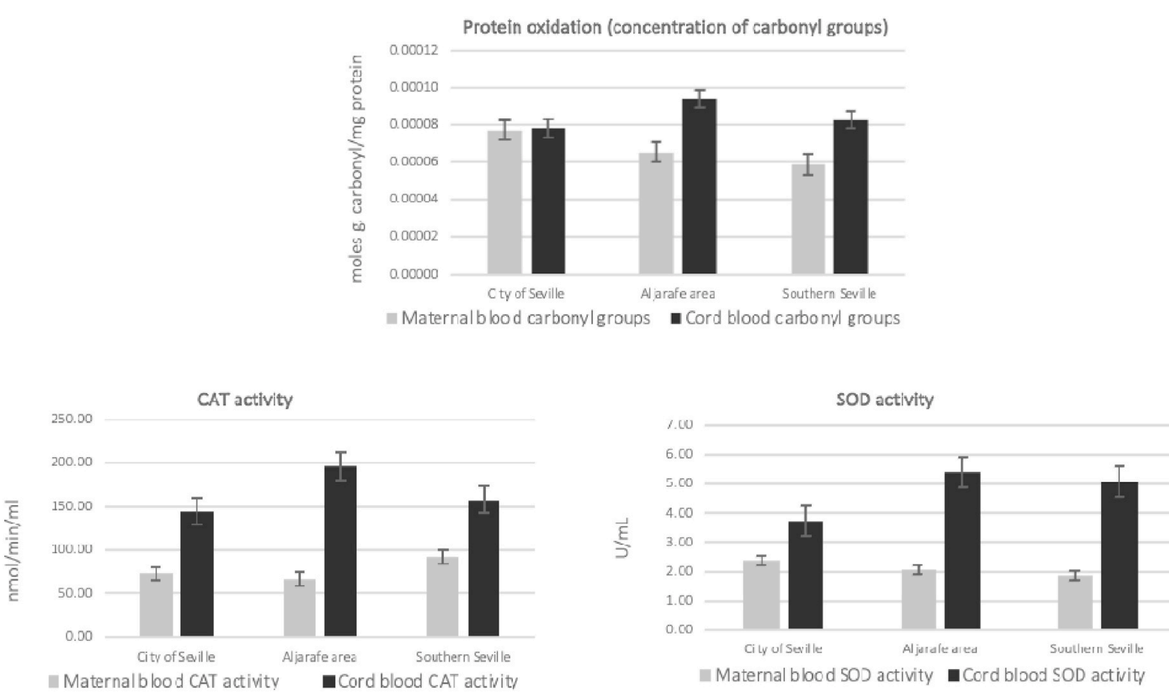


**Fig. 4.** Results obtained for all oxidative stress biomarkers and antioxidant levels. **A)** Median values for protein oxidation in maternal and cord blood of the three areas (SC, AA, and SSA) in moles g. carbonyl/mg protein. **B)** Median values for catalase activity in maternal and cord blood of the three areas (SC, AA, and SSA) in nmol/min/mL. **C)** Median values for SOD activity in maternal and cord blood of the three areas (SC, AA, and SSA) in U/mL. **: $p = 0.001$.

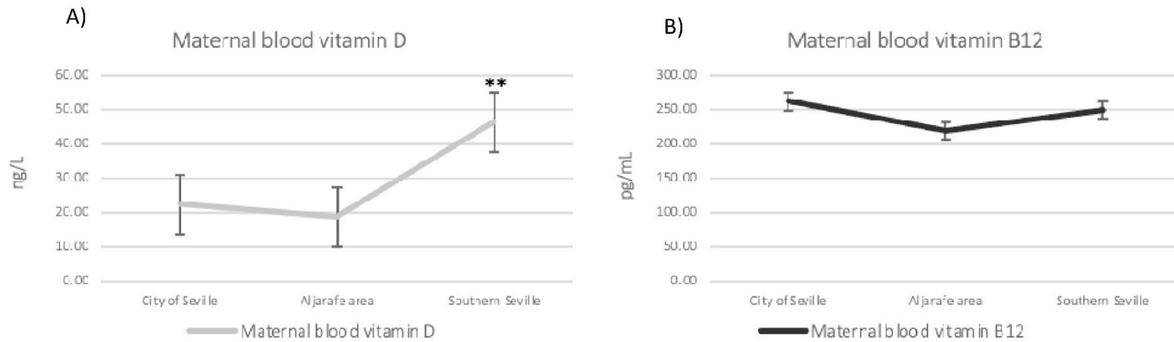


**Fig. 5.** Results obtained for vitamin D and B12 levels. **A)** Median values for vitamin D in maternal blood for the three areas (SC, AA, and SSA) in ng/L. **B)** Median values for vitamin B12 in maternal blood for the three areas (SC, AA, and SSA) in pg/mL. **: p = 0.001.

**Table 3**
Values of Cu, Se and Zn found in maternal and cord blood samples in the different areas.

| | | Maternal Cu (μg/L) | Cord blood Cu (μg/L) | Maternal Se (μg/L) | Cord blood Se (μg/L) | Maternal Zn (μg/L) | Cord blood Zn (μg/L) |
|---|---|---|---|---|---|---|---|
| **City of Seville** | **Median** | 1643.00 | 647.66 | 119.78 | 114.48 | 5077.40 | 2087.67 |
| | **Min** | 604.20 | 537.42 | 94.34 | 82.68 | 1632.40 | 1484.00 |
| | **Max** | 2173.00 | 1409.80 | 184.44 | 174.90 | 6890.00 | 7208.00 |
| **Aljarafe area** | **Median** | 1462.80 | 614.80 | 118.93 | 127.20 | 5088.00 | 2151.80 |
| | **Min** | 1250.80 | 552.26 | 59.36 | 109.18 | 4176.40 | 1653.60 |
| | **Max** | 1971.60 | 753.66 | 187.36 | 152.93 | 29150.00 | 2752.82 |
| **Southern Seville** | **Median** | 1633.46 | 657.20 | 116.60 | 113.42 | 5300.00 | 2268.40 |
| | **Min** | 604.20 | 293.62 | 0.00 | 38.16 | 0.00 | 921.14 |
| | **Max** | 2262.04 | 1515.80 | 159.00 | 185.58 | 24038.68 | 6105.60 |

**Table 4**
Correlation statistics of oxidative stress markers levels found in maternal and cord blood from Seville.

| | Maternal blood carbonyl groups (moles g. carbonyl/mg protein) | Cord blood carbonyl groups (moles g. carbonyl/mg protein) | Maternal blood CAT activity (nmol/min/mL) | Cord blood CAT activity (nmol/min/mL) | Maternal blood SOD activity (U/mL) | Cord blood SOD activity (U/mL) |
|---|---|---|---|---|---|---|
| **Shapiro-Wilk p*** | 0.001 | <0.0001 | 0.003 | <0.0001 | 0.13 | <0.0001 |
| **Pregnant women-child correlation (rho)** | 0.50 | 0.39 | 0.05 | | | |
| **Sig. (bilateral)** | <0.0001 | <0.05 | 0.75 | | | |

**Table 5**
Descriptive statistics of oxidative stress markers levels found in maternal and cord blood from Aljarafe area.

| | Maternal blood carbonyl groups (moles g. carbonyl/mg protein) | Cord blood carbonyl groups (moles g. carbonyl/mg protein) | Maternal blood CAT activity (nmol/min/mL) | Cord blood CAT activity (nmol/min/mL) | Maternal blood SOD activity (U/mL) | Cord blood SOD activity (U/mL) |
|---|---|---|---|---|---|---|
| **Shapiro-Wilk p*** | 0.13 | 0.005 | 0.003 | 0.004 | 0.01 | 0.41 |
| **Pregnant women-child correlation (rho)** | 0.37 | | 0.56 | | −0.35 | |
| **Sig. (bilateral)** | 0.06 | | <0.05 | | 0.18 | |

**Table 6**
Descriptive statistics of oxidative stress markers levels found in maternal and cord blood from Southern Seville.

| | Maternal blood carbonyl groups (moles g. carbonyl/mg protein) | Cord blood carbonyl groups (moles g. carbonyl/mg protein) | Maternal blood CAT activity (nmol/min/mL) | Cord blood CAT activity (nmol/min/mL) | Maternal blood SOD activity (U/mL) | Cord blood SOD activity (U/mL) |
|---|---|---|---|---|---|---|
| **Shapiro-Wilk p*** | <0.0001 | 0.0001 | <0.0001 | <0.0001 | 0.428 | 0.046 |
| **Pregnant women-child correlation (rho)** | 0.48 | | 0.37 | | −0.06 | |
| **Sig. (bilateral)** | <0.0001 | | <0.05 | | 0.69 | |

inside the moderate range, with a value of $\rho = 0.48$ ($p < 0.05$, establishing a range for ρ between 0.36 and 0.58 with a 95% confidence).

In contrast, all the weakest Spearman's correlations between maternal and cord blood presented not significant p values. In SC and SSA, the weakest correlations were observed in SOD activity, positive for the SC cohort, with values of $\rho = 0.05$ ($p = 0.75$, stating a range for ρ between −0.09 and 0.19 with a 95% confidence) and negative for the SSA cohort, with $\rho = -0.06$ ($p = 0.70$, ranging from −0.20 to 0.08 with a 95% confidence), respectively. In AA cohort, the weakest and negative correlation corresponded to SOD activity, with a value of $\rho = -0.35$ ($p = 0.18$, stablishing a range for ρ between −0.47 and −0.22 with a 95 % confidence).

Spearman's correlation statistics for metals in maternal and cord blood are presented in Table 7. The correlations observed were sometimes similar, in the case of Se and Cu and quite different for Zn between the three established cohorts. The strongest correlation was observed in maternal and cord blood levels of Cu in the AA with values of $\boldsymbol{\rho} = 0.53$, being inside the moderate range. The correlations were positive and weak to moderate in all Cu correlations for the different cohorts, negative and weak in the case of all different Se correlations for the three cohorts and a combination in the case of Zn, negative and moderate to weak for the AA and SSA cohorts.

Study of correlation between maternal and cord blood OS markers, enzymatic activities, metals, vitamins and sociodemographic characteristics.

Different possible correlations were studied between the following parameters: protein oxidation, as OS biomarker, CAT and SOD activity, as antioxidant enzymes and sociodemographic characteristics such as smoking habits, fruit and vegetables consumption and fish intake or pesticides use.

Regarding smoking habits and carbonyl groups on both maternal and cord blood, no statistically significant differences were obtained between the smokers and non-smokers. We also tried to establish a correlation between measured Cu values and smoking habits, but no statistically significant differences were obtained for this case, either.

Correlations between the numbers of fruits and vegetables consumed per day and CAT and SOD activity were studied, obtaining significant p-values for CAT maternal activity and vegetable consumption ($p = 0.04$) (Table 8).

A correlation was also pursued between the consumption of fruits and vegetables and the levels of Cu and Se found in both maternal and cord blood samples, but no statistically significant differences were found.

A possible correlation between consumption of ecological fruits and vegetables and these enzymes was also pursued, not finding differences on these antioxidant enzymes for those women who answered positively to using ecological products.

Weekly fish consumption can also be related to these antioxidant enzymes, so a correlation was established between the numbers of fish pieces consumed per week and CAT and SOD activity in both maternal and cord blood leading to non-significant p values in any of them.

**Table 8**
Correlation between the number of fruits and vegetables consumed per day and CAT and SOD activity.

| | Maternal blood | | Cord blood | |
|---|---|---|---|---|
| **Daily fruit consumption** | **CAT activity (nmol/min/mL)** | **SOD activity (U/mL)** | **CAT activity (nmol/min/mL)** | **SOD activity (U/mL)** |
| Spearman's rho<br>Sig. (bilateral) | −0.08<br>0.40 | 0.05<br>0.59 | −0.17<br>0.09 | 0.05<br>0.62 |
| **Daily vegetable consumption** | **CAT activity (nmol/min/mL)** | **SOD activity (U/mL)** | **CAT activity (nmol/min/mL)** | **SOD activity (U/mL)** |
| Spearman's rho<br>Sig. (bilateral) | −0.19<br>0.04 | 00.17<br>0.05 | 0.12<br>0.22 | −0.13<br>0.17 |

**Table 9**
Correlation between weekly seafood consumption and Cu and Se in maternal and cord blood.

| | Maternal blood | | Cord blood | |
|---|---|---|---|---|
| | Cu (μg/L) | Se (μg/L) | Cu (μg/L) | Se (μg/L) |
| Spearman's rho<br>Sig. (bilateral) | −0.24<br><0.05 | 0.01<br>0.90 | −0.14<br>0.25 | 0.23<br>0.85 |

A correlation between the weekly consumption of seafood and Cu and Se values in both maternal and cord blood was established (Table 9), finding statistically significant values for seafood consumption and Cu values in maternal blood samples.

For pesticides used on the household, we found significant differences for carbonyl groups values on both maternal and cord blood and the use of insecticides on their homes. However, no significant differences were found for p values between the oxidative stress biomarker and the use of herbicides on plants at home.

A correlation for Cu values in both blood samples and both pesticides and insecticide use in the household was also established but no statically significant values were found. For Zn and Cu values, since they have a close relationship with carbonyl groups, a correlation was also established finding no statistically significant values, either.

On the other hand, Se is more related to antioxidant enzymes, so correlations were established between these values in both maternal and cord samples and CAT and SOD activity (Table 10). Only significant different values were found for the established correlation between maternal Se values and SOD activity in the cord.

For vitamin D and B12 values, we also tried to establish relationship between them and nutritional characteristics that have been mentioned: vegetables and fruit daily consumption and weekly consumption of rice, fish and seafood. In this case, the only significant differences found were

**Table 7**
Descriptive statistics of Cu, Se and Zn levels found in maternal and cord blood from Seville, Aljarafe Area and Southern Seville.

| | | Maternal Se (μg/L) | Cord Se (μg/L) | Maternal Cu (μg/L) | Cord Cu (μg/L) | Maternal Zn (μg/L) | Cord Zn (μg/L) |
|---|---|---|---|---|---|---|---|
| **City of Sevilla** | **Shapiro-Wilk p*** | 0.01 | 0.07 | 0.05 | <0.0001 | 0.05 | 0.001 |
| | **Pregnant women-child correlation (rho)** | −0.05 | | 0.26 | | 0.31 | |
| | **Sig. (bilateral)** | 0.82 | | 0.27 | | 0.18 | |
| **Aljarafe Area** | **Shapiro-Wilk p*** | 0.90 | 0.16 | 0.17 | 0.01 | <0.0001 | 0.05 |
| | **Pregnant women-child correlation (rho)** | −0.20 | | 0.53 | | −0.48 | |
| | **Sig. (bilateral)** | 0.58 | | 0.12 | | 0.16 | |
| **Southern Sevilla** | **Shapiro-Wilk p*** | 0.14 | 0.06 | 0.05 | <0.0001 | 0.18 | <0.0001 |
| | **Pregnant women-child correlation (rho)** | −0.24 | | 0.15 | | −0.19 | |
| | **Sig. (bilateral)** | 0.26 | | 0.50 | | 0.39 | |

**Table 10**
Correlation between Se values and CAT and SOD activity.

| | Maternal blood | | Cord blood | |
|---|---|---|---|---|
| **Maternal blood Se (μg/L)** | **CAT activity (nmol/min/mL)** | **SOD activity (U/mL)** | **CAT activity (nmol/min/mL)** | **SOD activity (U/mL)** |
| Spearman's rho | −0.14 | −0.23 | 0.07 | −0.62 |
| Sig. (bilateral) | 0.28 | 0.31 | 0.57 | 0.03 |
| **Cord blood Se (μg/L)** | **CAT activity (nmol/min/mL)** | **SOD activity (U/mL)** | **CAT activity (nmol/min/mL)** | **SOD activity (U/mL)** |
| Spearman's rho | −0.13 | −0.12 | 0.13 | −0.28 |
| Sig. (bilateral) | 0.34 | 0.61 | 0.36 | 0.38 |

between vitamin B12 values in maternal blood and fish consumption.

Study of correlation between maternal and cord blood OS markers, enzymatic activities, metals, vitamins and newborn's characteristics.

Correlations were also studied between the measured parameters: protein oxidation, as oxidative stress biomarker, CAT and SOD activity, as antioxidant enzymes and the newborns' characteristics such as newborn's weight, height and head circumference.

Regarding the characteristics of the newborn, we could find significant difference between the antioxidant enzymes and the newborn's weight, specifically for SOD activity in maternal blood (Table 11).

Nonetheless, we could not find any statistically significant differences between the height and head circumference of the newborns and the protein oxidation as OS biomarkers, CAT, and SOD activity.

We also established relationships between vitamin D and B12 values and the newborn's characteristics, finding statistically significant difference for vitamin D values and the newborns height (Table 12).

## 4. Discussion

There are only a few studies about prenatal oxidative status [43,44], being this the first one performed in a cohort of Sevillian pregnant women, where levels of all the biomarkers measured presented a tendency to be higher in cord than in maternal blood, although non statistical difference was found. The strongest correlation in OS biomarkers between maternal and cord blood was observed in CAT in AA and for carbonyl groups in the SC and SAA groups. Regarding correlations between the OS markers and sociodemographic characteristics, only significant differences for carbonyl groups values were found on both maternal and cord blood, and the use of insecticides at home. Also, when the correlation between prenatal OS and newborn's characteristics was studied, only a significant correlation was detected between antioxidant enzymes and the newborn's weight, especially for SOD activity. For a study developed in South America, Brazil, some authors have found a correlation between CAT levels and complications in pregnancies, establishing a decrease in the activity of this enzyme in the third trimester for women with complications in comparation to those who had no complications [45]. These results could support the theory of higher risk of complications in the last week of pregnancy for those women who have higher OS status.

**Table 11**
Correlations between antioxidant enzymes (CAT and SOD activity) and newborn's weight.

| Weight (g) | Maternal blood | | Cord blood | |
|---|---|---|---|---|
| | **CAT activity (nmol/min/mL)** | **SOD activity (U/mL)** | **CAT activity (nmol/min/mL)** | **SOD activity (U/mL)** |
| Spearman's rho | 0.01 | −0.19 | 0.02 | −0.18 |
| Sig. (bilateral) | 0.93 | <0.05 | 0.85 | 0.05 |

**Table 12**
Correlations between vitamin D and b12 values and newborn's height.

| | Vitamin D | Vitamin B12 |
|---|---|---|
| Spearman's rho | 0.15 | 0.01 |
| Sig. (bilateral) | <0.05 | 0.87 |

Considering lifestyle, Caliri et al. (2021) [46] elaborated a review describing the powerful relationship between smoking and OS and other pathologies. In our study, although 16.70, 16.70, and 14.00 % of the pregnant women living in SC, AA, and SSA, respectively, declared being smokers before pregnancy, no significant correlations have been observed between these two factors.

Regarding protein oxidation, elevated carbonyl levels have been demonstrated to be related to high levels of OS [42]. A report developed in Poland [47] studied plasma carbonyl group concentration in pregnant women with intrauterine growth restriction, leading to 2.19 ± 0.67 mmol/mg protein in these pregnant women, and 1.90 ± 0.29 mmol/mg protein in the pregnant control group. Both values were higher than those described in our work, which ranged from 0.06 to 0.07 mmol/mg protein in maternal blood. However, Harma et al., (2004) [48] found a carbonyl group concentration of 1.40 ± 0.30 nmol/mg protein in healthy pregnant women, while in Algeria, also lower levels than ours were found on small, appropriate, and large for gestational age new-borns, being the appropriate for gestational age (1.37 ± 0.24 nmol/mg protein) the lowest [49]. Furthermore, the authors described that higher concentrations of carbonyl groups were related to a higher risk of protein destruction. In addition, Li et al., (2010) [50] described 10.00–40.00 μmol/mg protein in a cohort of both men and women, which is close to the results found in our study (59.00–73.00 μmol/mg protein).

About antioxidant enzymes, Bizoń et al. (2021) [51] analyzed the impact of early pregnancy and exposure to tobacco smoke on blood antioxidant status, and found a mean level of 47.3 ± 30.3 nmol/min/mL, and 1.5 ± 0.5 mU/mL SOD activity, much lower than the ones reported here (66.56–91.87 nmol/min/mL, and 2.38, 2.07, and 1.87 U/mL for SC, AA, and SSA, respectively). Furthermore, Tok et al. (2021) [52] described a remarkable difference in SOD activity between healthy pregnant (1.9 ± 0.5 U/mL) and ectopic pregnant (8.5 ± 1.9 U/mL) women, slightly elevated compared to our data. In contrast, the study carried out by Moore et al. (2020) [53] in pregnant women between 12 and 20 weeks of gestation, led to 8,600 U/mL, way higher than our results. This manifests the high variability in terms of SOD activity found in the literature. Even though there is high variability in terms of SOD activity found in the literature, some studies have also supported relationship between OS parameters, such as SOD and preterm birth. For example, Hamzaoğlu et al., (2023) found higher levels of SOD in the threatened preterm labour groups in a population of pregnant women from Istanbul, Turkey, against the control group [54]. They also found a negative correlation between SOD values and birth weight for all groups [54].

As for the measured metals, in the case of Cu, there was a consistent tendency of lower cord blood levels than maternal levels (maternal levels of 1643.00 μg/L, 1462.80 μg/L and 1633.46 μg/L vs 647.66 μg/L, 641.80 μg/L and 657.22 μg/L in cord blood). This was also found in other studies, one of them also performed in Spain, conducted in Tarragona [55] (1664 μg/L in maternal blood versus 623 μg/L in cord blood). Outside of Spain, in a cohort of pregnant women from a University Hospital in China, Gu et al. (2022) [56] also found lower levels for cord blood in Cu, 249.98 μg/L against the 1844.87 μg/L in maternal blood. Authors have stated that low levels of Cu could be predictors of some pathological pregnancies such as abortion, o premature membrane rupture [57], showing significant differences in these metal's levels in women who had history of these pathologies against control groups in a cohort of women from Turkey. For Cu some authors have found negative correlations between Cu values in cord blood and birth weight for the newborn [58,59].

For Zn, a similar tendency was followed, being the levels found in cord blood lower than the ones found in maternal blood in the three cohorts (5077.40 μg/L in SC, 5088.00 μg/L in AA and 5300.00 μg/L in SSA for maternal blood vs 2087.67 μg/L, 2151.80 μg/L and 2268.40 μg/L in cord blood, respectively for the different areas). These values were also the highest recorded for the different metals measured, and quite similar to the study performed in pregnant women in northern Spain [60] with values of 6708 μg/L for maternal and 2311 μg/L for cord blood. Also, Lozano et al., (2022) [61] found out that these metal was also the highest throughout all pregnancy in urine samples for the Valencia, Gipuzkoa and Sabadell cohorts in the INMA Project.

Se levels were the only ones not following the tendency that has been stated, being maternal and cord values quite similar, ranging from 111.78 to 123.07 μg/L for both types of samples in the different cohorts. Se levels are important to assure an appropriate antioxidative protection for the foetus, having a higher demand in pregnancy due to an increase of erythrocyte mass [57], that could explain the positive correlation we found between these maternal metal levels and SOD levels in cord blood. Some authors [60] have also found a correlation between these metal's levels and some dietary characteristics, such as seafood, which was positive and negative for fruits, that we could not find.

As for the vitamin levels, vitamin D values found in our cohort were lower than 50 nmol/L which is the established normal level in pregnancy, being the values for the AA the lowest ones with a median value of 18.80 nmol/L, and 46.40 nmol/L for the SSA women the highest, and 22.50 nmol/L for the SC pregnant women. Díaz-López et al., (2020) [62] also found lower values for their cohort in Tarragona, northern Spain, with a mean value of 33.9 nmol/L (SD, 17.0) during the whole pregnancy. As stated by them, these values could be related to the hours of sun in each location, being lower for the north of the country in comparison to the south [62] but our values showed lower levels for the AA and SC cohort. As for the positive correlation we found between vitamin D levels and the new-born's height, maternal vitamin D levels have always been associated with the offspring bone mass and immunity, which could induce small for gestational age (SGA) new-borns. Many studies have proposed that vitamin D supplementation should be a basic care recommendation [63,64], like it has been done for vitamin B12, but for all women and more especially pregnant and those in child-bearing age.

Vitamin B12 has been associated with adverse infant health outcomes and is closely monitored during pregnancy due to them. For our three cohorts, B12 levels are quite similar, being the ones for SC the highest (261.50 pg/mL). A study conducted in Catalonia, Spain, inside the ECLIPSES Study, obtained B12 values higher than the ones we found for both the 1st and 3rd trimester for pregnancy (374.2 ± 127.7 pg/mL and 305.2 ± 138.0 pg/mL) [65] also establishing a close relationship to the maternal vitamin values and different characteristics of the new-born such as better motor, cognitive, and language performance 40 days after birth.

## 5. Conclusions

In summary, all biomarkers measured in the present study are within normal ranges for maternal and cord blood values and like the ones found by other authors globally. Since all the values obtained presented a tendency to be higher in cord blood than maternal blood, although non statistical difference was found, this might suggest a higher metabolism in cord blood, implying a higher production of ROS and, therefore, a higher production of antioxidant enzymes to maintain a normal balance. Regarding the different cohorts, our results led to no significant differences between all pregnant women in our study.

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## Author contribution

Conceptualization: Lucas Cerrillos, Rosa Ostos, and Isabel Moreno; methodology: María Hinojosa, Pilar Carbonero-Aguilar, and Bouchra Dahiri; resources: Isabel Moreno; writing—original draft preparation: Bouchra Dahiri and María Hinojosa; writing—review and editing: Juan Bautista, María Hinojosa, and Isabel Moreno.

## Declaration of competing interest

The authors declare no conflict of interest.

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