Potential role of p53 protein as a novel biomarker of sperm quality, able to predict the success of ART techniques. EcoFoodFertility Project

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**Introduction:** Protein p53 is well known as “The guardian of genome”; it changes its concentration in human spermatozoa DNA in relation to the damage of the latter. It has been suggested that the role of the p53 ancestral gene was to ensure the integrity of the genomic germline and the fidelity of the development process. The aim of this study is to evaluate if different concentrations of p53 protein in human spermatozoa could influence embryo quality and pregnancy rate and possibly representing a potential predictive marker of sperm quality for successful fertilization.

**Methods:** From July 2013 to June 2017 we have examined retrospectively 79 couples with 2–5 years of infertility history. Males had an average age of 27±7.5 years, sperm concentration of 33.8±6.2 mil/ml, progressive motility of 41.4±8.3 and a typical morphology of 16.5±3.5 according to Kruger’s method. We have divided the couples on the basis of p53 levels: Group A: 0.35–1.65 ng/ml (21 males); Group B: 1.66–3.57 ng/ml (32 males); Group C: 3.58–14.53 ng/ml (26 males). We have evaluated the number of embryos at stage of 6–8 cells, obtained at the third day of embryo development, in these three different group. In order to evaluate the concentration of p53 protein, we first proceeded to a DNA extraction with forensic method and then to a quantitative determination of p53 protein. After 3 months without taking medicine we proposed to the participants to seminal fluid analysis to evaluate the p53 protein concentration. After 3 months without taking medicine that could influence the state of semen, we proposed to members of the group B and C to take part to a new observational study integrating to the principal meals (breakfast, lunch and dinner) 400 mg of alpha- tocoferol for 30 days. After 30 days we subjected the participants to seminal fluid analysis to evaluate the p53 protein concentration. After 3 months without taking medicine that could influence the state of semen, we proposed to members of the group B and C to take part to a new observational study integrating to the principal meals (breakfast, lunch and dinner) 400 mg of alpha- tocoferol for 30 days. After 30 days we subjected the participants to seminal fluid analysis to evaluate the p53 protein concentration. After 3 months without taking medicine that could influence the state of semen, we proposed to members of the group B and C to take part to a new observational study. The role of synergic action between alpha-Tocopherol and lifestyle on reduction of p53 protein in human spermatozoa.

**Introduction:** Oxidative stress has been identified as one of the many mediators of male infertility by causing sperm dysfunction. The aim of this study within the preliminary diet strategies of EcoFoodFertility project is to evaluate the variations of p53 protein after the introduction of an antioxidant in the daily diet and changing the life-style.

**Methods:** We recruited 45 male participants (age 28.0±5.6) with sperm concentration (4 to 50 Million/Milliliter), Motility (2 to 3+), morphology upper 14%. The spermatozoa DNA damage were evaluated on the quantitative determination of p53 protein. We realized a DNA extraction with forensic method and a successive quantification with ELISA-immunoenzimatic. The values were expressed in ng/Million of spermatozoa. The participants were divided in 3 groups according to the different p53 protein concentration at time 0': Group A, 20 patients with [p53] included between 3,68 and 5,7; Group B, 14 patients with [p53] included between 6,0 and 10,96; Group C, 11 patients with [p53] included between 11,02 and 17,85. We proposed to the participants to integrate to the principal meals (breakfast, lunch and dinner) 400 mg of alpha- tocoferol for 30 days. After 30 days we subjected the participants to seminal fluid analysis to evaluate the p53 protein concentration. After 3 months without taking medicine that could influence the state of semen, we proposed to members of the group B and C to take part to a new observational study adding the alpha-Tocopherol in the diet for 30 days with indications about life style regarding have meals at regular time, eliminating some food and drinks that have a strictly contact with plastic and a moderate physical activity 2–3 times a week.

**Results:** After 30 days: Group A: values at time 0' of 5,69±2,01, values after 30 days 1,25±0,4 with a reduction equal as 71,01%. Group B: values at time 0' of 8,48±2,48, values after 30 days 4,71±2,16 with a reduction equal as 42,11%. Group C: values at time 0' 14,43±3,41, values after 30 days 12,11±2,87 with a reduction equal as 14,16%. Using the method of the first study, after 3 months: Group B: new determination of [p53] at time 0' is 8,57±2,45, values after 30 days 3,29±2,02 with a reduction equal as 59,5%. Group C: new determination of [p53] at time 0' is 14,15±2,84, after 30 days 11,01±2,77 with a reduction equal as 23,30%.

**Conclusions:** Introducing alpha-Tocopherol in the diet, we have a substantial decrease of p53 in the group “A”. Adding advices to the life-style of groups “B” and “C”, reduction of p53 is more marked and, in some cases, values are normalized. The synergic

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Lastly, sperm selection for assisted reproduction (ART) according to content of sex chromosomes will be evaluated. 2. Selection Mechanisms within the Female Reproductive Tract. At ejaculation, billions of spermatozoa are released into the female reproductive tract and start to make their way to the site of fertilization. Some individuals produce ejaculates that are better able to withstand cryopreservation than others, that is, with higher cryosurvival. In the semen industry, it is usual to select animals that are “good” freezers as breeding sires, since the market for straws of frozen semen with poor viability is nonexistent. Results: Immunohistochemistry showed elevation of p53 protein level in 77% of the cases, while RT-PCR showed downregulation of p53 mRNA and its seven target genes in 23% and 47-97% of the samples. PAI-1 was down-regulated in almost all CCA samples, thus highlighting it as a potential diagnostic marker for CCA. The above mention associations of S100A9 and WIP1 expressions with clinicopathological features suggest their potential biomarkers of CCA progression, but the number of cases are too limited to make any conclusion. It is of note that PAI-1 was down-regulated in 97% of CCA tissue samples, thus highlighting its loss in the etiology of CCA and its potential diagnostic marker of CCA. Proteins can be denatured by agents such as heat and urea that cause unfolding of polypeptide chains without causing hydrolysis of peptide bonds. The denaturing agents destroy secondary and tertiary structures, without affecting the primary structure. If a denatured protein returns to its native state after the denaturing agent is removed, the process is called renaturation. These techniques improve quantitative analysis of the â€“ followed by separation by mass in conventional laemmli gels identified proteins as well as reproducibility of the data analyses and staining. Each protein thus forms a spot on a gel, and spot intensities are compared across gels in order to observe differ. Another important point is that coverage of the proteome ential expression levels. Another study also found this major sperm biomarker for diagnosis and prognosis of male factor infertility component to be underexpressed in oxidatively stressed â€“ thus helping to develop an appropriate therapeutic spermatozoa [101]. Volume 75 Issue 2. The potential application of a biomarker approach for English FranÃ§ais. Proceedings of the Nutrition Society. It will also explore the possibility of implementing a novel biomarker approach for investigating LCS exposure, which could be used to gain a better understanding of LCS consumption and health. Low-calorie sweeteners. The focus of this review will be on nutritional biomarkers of exposure and within this specific context, biomarkers will be defined as cellular, biochemical, analytical, or molecular measures that are obtained from biological media such as tissues, cells or fluids and are indicative of exposure to an agent( 39 ).