

## PAPER

# A Virtual Reality Game to Promote the Role of a Healthy Diet in Male Reproduction

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## ABSTRACT

This paper presents gaming as an alternative way of transmitting information compared to traditional academic methods. New technologies provide an opportunity, and in particular, virtual reality gaming allows users to have an immersive, personifying, intuitive, and engaging experience useful in visualizing complex concepts. Questionnaires were administered to secondary school students to extrapolate relevant information regarding the role of diet on sperm quality following the experience of the virtual reality game “Oxistress.” This game allows participants to understand in a playful and recreational way how the presence of reactive oxygen species can damage spermatozoa and how adequate diet choices provide ammunition in the form of antioxidants and omega-3 fatty acids to protect and repair spermatozoa. The results suggest that virtual reality (VR) may represent an important way to improve knowledge of scientific topics. Further studies will be necessary, involving larger populations with different basic knowledge as well as checks on whether the information acquired remains in memory after months.

## KEYWORDS

diet, learning, omega 3, reactive oxygen species, sperm quality, virtual reality (VR)

## 1 INTRODUCTION

Virtual reality (VR) is an innovative tool that, due to its immersive and multisensory nature, can fulfill the principles of active learning. The immersive experiences it offers and the sense of presence and embodiment are all key factors that can promote knowledge acquisition [1]. VR offers three main opportunities: it can convert the abstract into the tangible, it supports doing rather than just observing, and it can replace learning methods that would be ideal but have little application in reality [2]. Especially when applied to studies related to health and nutrition education [3], we can say that games can be equally, if not more, effective than traditional learning methods [4].

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In recent years, various studies have shown a decrease in sperm quality, which can be linked to increased exposure to chemicals such as pesticides, plastics, and other anthropogenic materials [5]. Additionally, lifestyle factors such as smoking habits [6] and/or excessive alcohol consumption can affect sperm production and quality [7]. Over the last two decades, sperm concentration has exhibited a further progressive decline, and a significant decrease in fertilization rates has been hypothesized over the next 30 years [8].

Dietary patterns, the components of diet, and nutrients have been studied as possible determinants of sperm function and fertility [9, 10]. Anti-inflammatory diets, such as the Mediterranean diet (which includes increased intake of monounsaturated and n-3 polyunsaturated fatty acids, flavonoids, and reduced intake of red and processed meat), have been shown to improve fertility, assisted reproductive technology (ART) success, and sperm quality in men [11].

In men, the ability to modulate inflammatory processes and reactive oxygen species (ROS) formation through dietary and non-pharmacological treatments may be essential to improving male reproductive outcomes. Unfortunately, humans cannot serve as an ideal model for study due to the significant variability in genetics, behavior, and lifestyles. Animal models can be beneficial in research.

Our group obtained scientific data on the effect of an omega-3 diet on rabbit sperm and testes [12]. This data was used to conduct a VR experiment.

The VR game “Oxistress,” developed by the Virtual Reality Laboratory (LabVR) of the Department of Political, Social, and Cognitive Sciences in collaboration with the Department of Molecular and Developmental Medicine at the University of Siena, has made it possible to visualize the impact of proper nutrition on spermatozoa. The game demonstrates, in a playful and recreational manner, how the presence of ROS can harm sperm and how making appropriate dietary choices can supply antioxidants and omega-3 fatty acids to safeguard and restore sperm. The concept is to educate through interactive gameplay [13].

The aim of this study was to assess the ability of a group of students to acquire scientific information while using Oxistress.

## 2 MATERIALS AND METHODS

### 2.1 Participants

The application of virtual reality was tested on a sample of 30 secondary school students (aged 17–19) from the Technical Technological Institute and High School of Applied Sciences “Tito Sarrocchi” in Siena. The sample consisted of 14 males and 16 females. One female student was excluded from the study due to anxiety issues related to the experience. Prior to participation, the students’ parents provided consent for their children to take part in the project. All anti-Covid procedures recommended by the Prevention and Protection Service of the University of Siena were adhered to.

## 2.2 Experimental protocol

The experience took place in March 2023 at the Santa Chiara Lab of the University of Siena in LabVR. Two areas were arranged for the experiment. The first area was furnished with four VR game stations, each equipped with the Oculus Quest 2 visor (Meta, Menlo Park, California, United States), and a computer or tablet for researchers to assist students during the simulation by observing the game. The second area was designated for completing questionnaires regarding the experience.

Before the VR session began, a brief introductory talk was given about the global trend of male infertility in the world and the VR experience that the students would undergo. Subsequently, each participant was led through the game simulation. It took between 30 and 40 minutes to complete the entire simulation.

During the game, it is possible to open information panels on the various biological topics covered.

Oxistress is a narrative game designed to educate a non-specialized audience about the biological process of oxidative stress and the roles of free radicals, antioxidants, and fatty acids in either accelerating or limiting it. By engaging with the game, players can understand the impact of dietary habits on the system, especially on the quality of the seminal fluid, and overall reproductive health.

In Oxistress, the player helps a group of astronauts make optimal dietary choices to maintain their fertility levels and ensure the success of the colonization mission.

The science-fiction setting provides a narrative strategy to isolate the system you want to explain and maximize agency, creating a sensible context for the association of individual game choices (such as what to eat) with immediate macroscopic consequences (such as the effect on the fertility of the crew).

The game was developed by the LabVR UNSI team using the Unity game development engine and runs on modern VR headsets such as Meta Quest. Here follows a link to a short video introducing the VR game: <https://www.youtube.com/watch?v=JgRwJ4UaP7E>

Two questionnaires were designed to evaluate the students' learning. They were completed anonymously, one before playing the game and one after the simulation was conducted. The pre-experience questionnaire aimed to understand the participants' initial knowledge in the fields of nutrition and reproductive biology (see Figure 1). In contrast, the post-experience questionnaire aimed to assess the degree of specific knowledge achieved and new information acquired in the fields of reproductive biology and nutrition through specific questions about what had been presented in the game (see Figure 2). Additionally, LabVR researchers administered a questionnaire to evaluate the virtual reality simulation itself. This aimed to understand the level of engagement, enjoyment of the game, and any feelings of dizziness or nausea to enhance the Oxistress game.

1. On a scale of 0 to 10 how much attention do you pay to your food choices?

0	1	2	3	4	5	6	7	8	9	10
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2. On a scale of 0 to 10 how much do you think food affects your overall health?

0	1	2	3	4	5	6	7	8	9	10
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3. Of these, which do you think are the principles of the Mediterranean diet?

- Consumption of fruit, vegetables, legumes, whole grains, dried fruit, extra virgin olive oil and fish
- Consumption of fruit, vegetables, poultry and red meat, dairy products, restricted bread and pasta
- Consumption of refined cereals, butter, margarine, few fruit and vegetables, preference of eggs and cold cuts as main course

4. Which of these foods do you think should be preferred because of its nutritional properties?

- Fish
- Red meat
- Matured cheeses (parmesan, pecorino, grana padano...)

5. Have you ever heard of saturated and trans fats?

- Yes
- No

6. Do you believe that your eating habits can affect your reproductive capacity?

- Yes
- No

7. Have you ever studied the spermatozoon?

- Yes
- No

8. Which of these statements do you think is correct?

- The spermatozoon consists of a part containing the nucleus called the head, an intermediate portion and a tail called the flagellum which allows movement
- They have a diploid genetic make-up, meaning they have two copies of genetic material for each chromosome
- They have a head containing the genetic material surrounded by a protein membrane

9. What do you think is a free oxygen radical (ROS)?

- Molecules with a stray electron produced by the body due to processes such as oxidative stress or normal oxidation-reduction reactions in the cell
- Highly reactive molecules involved in repairing cell damage
- Totally harmless waste products of the cell

10. Have you ever heard of antioxidants?

- Yes
- No

11. Given the proposed statements, what do you think is the correct definition of antioxidants?

- They are substances found inside the cell and that we can take in through food, which are able to inhibit oxidation processes that damage cellular material
- They are enzymes that enable the digestion of food containing oxygen molecules
- They are substances exclusively produced by the cell to fight the damage caused by the waste products of cell metabolism

12. Have you ever heard of omega-3?

- Yes
- No

13. In your opinion, which group of macromolecules do omega-3s belong to?

- Fats
- Protein
- Carbohydrates

Fig. 1. Pre-experience questionnaire

1. **Based on your experience, which of the following do you think is the correct definition of a free radical?**
  - These are highly reactive molecules involved in repairing damage to the phospholipid membrane of the spermatozoa
  - Waste products of the cell that help counteract the production of antioxidants
  - They are molecules with an unbalanced electron produced by our organism due to processes such as oxidative stress or normal oxidation-reduction reactions of the cell that damage the phospholipid membranes of spermatozoa
2. **Which of these are the parameters that make it possible to understand if the spermatozoon is healthy and efficient?**
  - Vitality, morphology, motility
  - Strength, antioxidant content, head size
  - Tail length, vitality and potency
3. **Based on your experience, to counteract free radicals, what would you try to assume through your diet?**
  - Protein
  - Antioxidants
  - Fatty acids
4. **Based on your recent experience, which of the following can be defined as antioxidants?**
  - Nitrosamines, heterocyclic amines and ethanol
  - Insulin, glucagon and somatostatin
  - Carotenoids, polyphenols, flavonoids, tocopherols and vitamin C
5. **Why are fatty acids relevant to sperm health?**
  - Because they represent the majority of the spermatozoon's plasma membrane
  - Because they increase tail motility
  - Because they counteract the action of free radicals
6. **Based on your experience, which of the following are the most important fatty acids for sperm health?**
  - Monounsaturated fatty acids
  - Polyunsaturated fatty acids (omega 3 and omega 6)
  - Saturated fatty acids
7. **Where are omega 3 fatty acids found most?**
  - Poultry and olive oil
  - Eggs, milk and milk products (yoghurt, fresh cheese, mature cheese)
  - Oily fish, dried fruit and green leafy vegetables

Fig. 2. Post-experience questionnaire

### 3 RESULTS

The aim of the questions in the pre-experience questionnaire was to assess preliminary and general knowledge about nutrition and reproductive biology. The first two questions required students to rate, on a scale of 0 to 10, the level of attention they personally devote to their food choices (question 1) and their belief in how much their health depends on a balanced diet (question 2).

The score that was most selected for the first question was 7, chosen by eleven students, while only one student gave a score of 10. Only two students were below the sufficient mark, indicating their attention to their food choices as 4. The total arithmetic mean of the scores is 7.32.

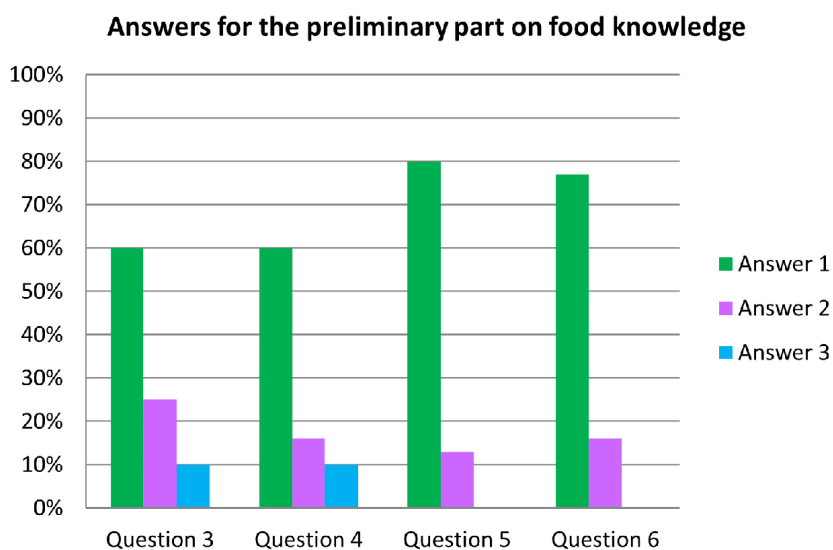
In the second question, however, most of the students ( $n = 25$ ) rated the importance of nutrition for good general health with a score of at least 8. No one rated it lower than sufficient, and only one student marked 6. The total arithmetic mean of the scores was 8.61.

The third question aimed to find out what the students knew about the Mediterranean diet. 62% of the students answered correctly (consumption of

fruit, vegetables, legumes, whole grains, dried fruit, extra virgin olive oil, and fish). A good percentage of students, 28%, thought it was correct to choose the option stating that one of the principles of the Mediterranean diet is to reduce bread and pasta.

In the fourth question, 69% of the students selected fish as the food with the best nutritional properties, compared to 20% who selected red meat and 11% cheese. Question four, as well as questions five and six, were not asked to obtain an unequivocal truth about food, as this does not exist. Instead, one must speak of a varied diet rather than “food” with the best nutritional properties. These questions were designed to assess the students’ level of previous knowledge on the subject. For example, the risk factor that red meat, when processed or consumed in excess, may represent for the development of cancer, or whether they were aware that mature cheeses, while rich in proteins of high biological value and minerals such as calcium, are also high in salt and saturated fatty acids and should therefore be limited in their daily intake. In the fifth question, students were asked whether they had ever heard about saturated fatty acids and trans-fatty acids; 83% said yes and 17% said no. To conclude the section on preliminary knowledge of nutrition, the sixth question asked whether there was, in their opinion, a correlation between eating habits and reproductive capacity.

The answer was yes in 79% of the cases and no in 21% (see Figure 3). The questions were then more specific and related to the topics covered in the game. These questions aimed to better understand the role of the virtual reality experience in increasing their knowledge. In question 7, which asked if students had ever studied the spermatozoon, 65% said yes, while 35% said no.



**Fig. 3.** Histogram showing the percentage of answers given to the questions on food knowledge in the pre-experience questionnaire

Students were then asked to choose one correct affirmation from the statements presented regarding the spermatozoon. 89% of the students selected the correct answer, while 11% gave one of the two incorrect answers. The ninth question concerning the definition of a free radical had 76% correct answers, while 20% of the students chose the answer that defined it as a molecule capable of repairing cell damage, and only one participant thought it was a totally harmless waste product of the cell. The tenth question asked if they had ever heard of antioxidants; an

affirmative answer was given by 89% of the students. This knowledge was further investigated by asking them to choose between several options for the correct definition of antioxidants. 79% of the students gave the correct answer: that antioxidants are found both inside the cell and that they can be obtained through food. 14% of the students answered that they are molecules exclusively produced by the cell and therefore could not be obtained through food.

In question twelve, 96% of the students claimed they were familiar with omega-3. However, when asked in question thirteen which group of macromolecules omega-3 belonged to, only 13 students (44%) correctly answered ‘fats,’ while 49% believed it was proteins, and 7% thought it was carbohydrates. The data is illustrated in the histogram (see Figure 4).

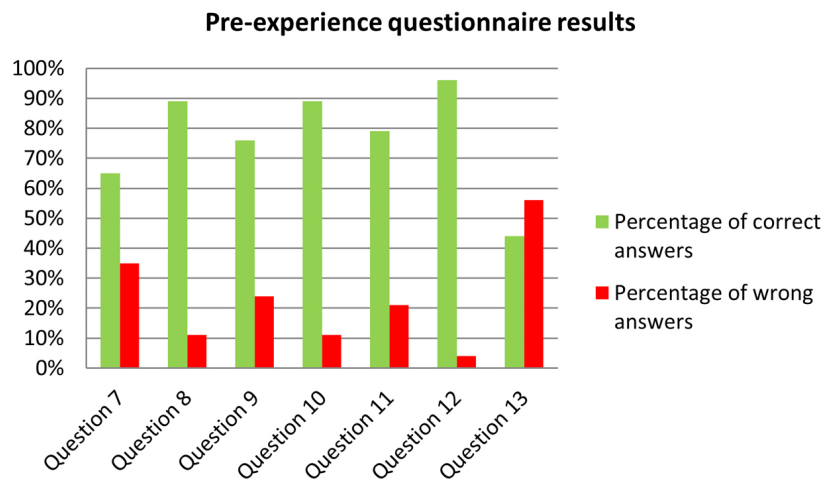


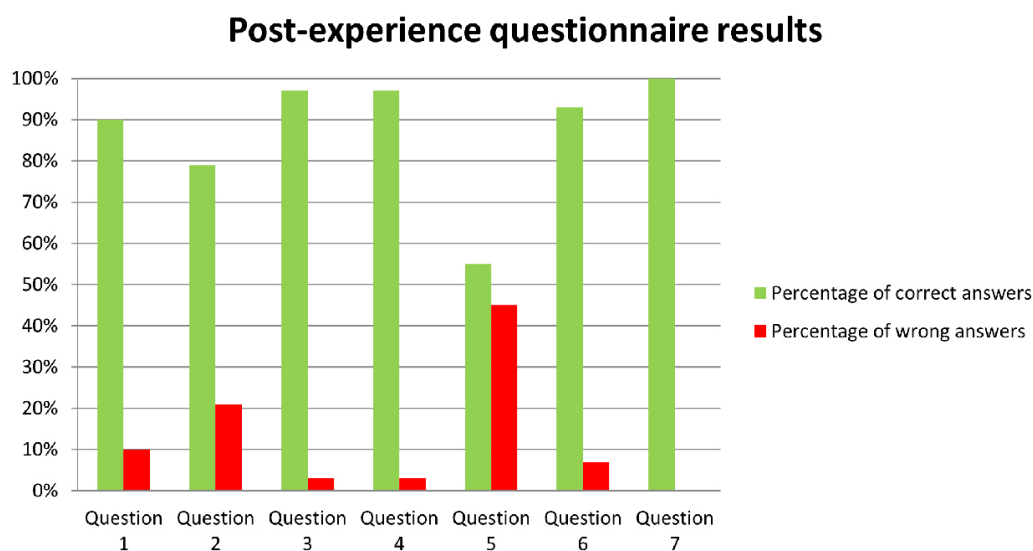
Fig. 4. Representative histogram of response rates to the pre-experience questionnaire

The post-experience questionnaire presented targeted questions about the topics shown in the game. The same questions from the pre-experience questionnaire were not repeated. Instead, an effort was made to use phrases very similar to those found in the explanatory panels of the virtual reality to evaluate how well they were read and understood. The first question reiterated the issue raised in the pre-experience questionnaire, which was the correct definition of a free radical. In this case, 90% of the responses were correct, with only three boys selecting an incorrect answer. This question already showed a more than 10% increase in correct responses. The percentage of correct answers regarding the definition of a free radical rose from 76% to 90%, representing an overall increase in 14% of correct responses. The second question inquired about the parameters that indicate the health status of a spermatozoon. 80% of the students provided the correct answer, while 17% considered an answer that also included the antioxidant content in the spermatozoon as correct. While this consideration may not be incorrect, since the qualitative parameters of the spermatozoon are explicitly displayed in the game and are criteria for progression, this answer was still deemed incorrect.

Only one student answered that the sperm parameters were tail length, vitality, and sperm potency. The third question, about what is correct to assume through food to counteract free radicals, received 97% correct answers. The correct answer was ‘antioxidants’; the other answers were proteins, which received no selection, and fatty acids, which were selected by only one student. All in all, fatty acids are not a completely wrong answer, but considering the definition as generic and

not specifying what kind of fatty acids, the answer is to be considered wrong. The fourth question also had a high percentage of correct answers (97%). It asked how an 'antioxidant' could be defined. Only one student stated that antioxidants are nitrosamines, heterocyclic amines, and ethanol. A question concerning the definition of an antioxidant had already been proposed in the pre-experience questionnaire and had received a 79% correct answer rate. There was therefore an increase of 18% in correct answers. The fifth question, perhaps the most ambiguous of the entire post-experience questionnaire, asked why fatty acids are important for sperm health. 55% answered that it is because they constitute most of the spermatozoon's plasma membrane, which is the correct answer, but 41% answered that they counteract the action of free radicals. In the game, this issue is raised, but we see that it is the omega-3 polyunsaturated fatty acids (PUFA) and not generic fatty acids, that counteract free radical damage. These latter play a role in membrane repair. The next question, the sixth, was supposed to clarify if the concept of omega-3 as the most important fatty acid for sperm health was understood. It asked which of the proposed fatty acids were the most important for sperm health, and 93% of the students answered omega-3 and omega-6 PUFA. Only two students answered saturated fatty acids. At last, when asked which were the main food sources of omega-3 PUFA, 100% of the students gave the right answer, that is, oily fish, dried fruit, and green leafy vegetables. Compared to the pre-experience questionnaire, where 49% of the students had answered that omega-3 belonged to proteins, in this question it can be observed that following the VR experience, the students reconnected the source of omega-3 to what they had been shown in the game, therefore not as 'proteins' but as components of foods consisting of even more macromolecules, then implicitly identifying omega-3 among the fatty acids in the sixth question.

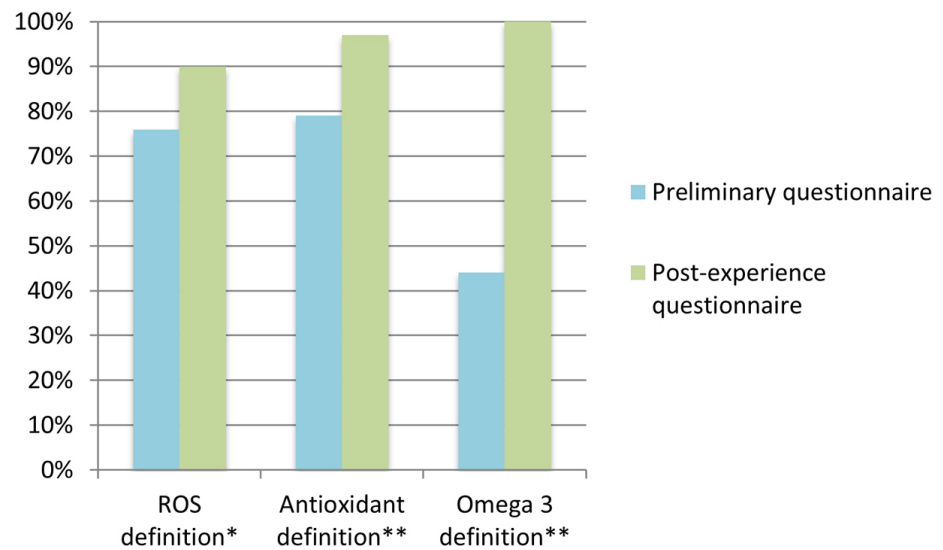
Data are shown in the histogram (see Figure 5).



**Fig. 5.** Representative histogram representing the response rates to the post-experience questionnaire

When the results of the pre-experience questionnaire are compared with those obtained in the post-experience questionnaire, correct answers were achieved in at least 90% of the questions, with the exception of one question that may have been more ambiguous than the others.





**Fig. 6.** Histogram comparing the correct answers with the same question topic in the pre-experience questionnaire versus the post-experience questionnaire

*Note:* An increase of at least 10% of the correct answers given after the virtual reality experience can be appreciated.  $P < 0.05$  was considered significant. \* $P < 0.05$ ; \*\* $P < 0.01$ .

When comparing questions with similar topics in the pre- and post-experience questionnaire, the number of correct answers significantly increased (ROS definition,  $p < 0.05$ , antioxidant and omega 3 definition  $p < 0.01$  (see Figure 6)). The data was compared using the z-test for the binomial proportion directly performed on Excel.

## 4 DISCUSSION

Male fertility is declining. Studies have shown that dietary and lifestyle habits are crucial determinants of sperm function and fertility. Therefore, it is important to disseminate science-based knowledge on how a healthy diet can prevent certain male infertility [14]. A comprehensive systematic review of observational studies [9] revealed that omega-3 fatty acids, antioxidants such as vitamin E, vitamin C,  $\beta$ -carotene, selenium, zinc, cryptoxanthin, lycopene, vitamin D, folate, and low levels of saturated fatty acids and trans-fatty acids were inversely associated with low semen quality. Fish, shellfish, seafood, poultry, cereals, vegetables, and fruits were positively correlated with sperm quality parameters. Negative influences were also observed for processed meat, soy foods, potatoes, high alcohol intake, and caffeine. Animal models could help identify dietary therapies for reproductive diseases [15]. Men's fertility is significantly affected by obesity, diabetes, varicocele, and infections characterized by chronic inflammation, oxidative stress, and insulin resistance. In these pathological conditions, an increased level of ROS is counterbalanced by antioxidant treatment [16].

Dietary fatty acid supplementation influences the modulation of sperm mitochondria energy metabolism and, in turn, determines the improvement of sperm quality in rats [17]. These data underscore the importance of providing information on proper nutrition as a lifestyle choice, especially to young people in primary and secondary schools. In this context, a virtual game was developed where making correct food choices supports game progression. This game was tested on high school students. The playful aspect of the simulation is combined with an educational component that educates players about which foods in their daily diet contain antioxidants and omega-3, enabling users to connect the dynamics of the game to real life [13]. Two questionnaires

were administered: the pre-experience questionnaire was designed to evaluate the children's knowledge of nutrition. The responses we received indicated that a significant percentage of students are mindful of their daily food choices. Moreover, 25 out of 30 students believed it was important to rate the significance of food for their overall health between eight and 10. This suggests that the students participating in the study had a good understanding of the role that daily nutrition plays in their well-being.

A question on the definition of the Mediterranean diet was voluntarily included; a good percentage of students reported that the Mediterranean diet would involve a reduction of bread and pasta. This could be explained by the demonization of carbohydrates by certain strict dietary protocols that have become increasingly popular on social media, such as the ketogenic diet. However, the scientifically defined Mediterranean diet, and the only diet approved even in the long term along with vegetarian diets for sustaining optimal health, includes a predominant presence in the daily diet of carbohydrates, derived from whole grains or from grains themselves in grain form, up to 55%–60% of the macronutrient composition of the day's diet. This data suggests that in food prevention for both primary and secondary school students, 'playful' components such as our experience in VR can be exploited to properly nourish our organism and to have a tool for understanding and filtering the most disparate information that is now readily available on social media and the Internet [18, 19]. Overall, the questions asked to understand the degree of biological knowledge and interest in food revealed that there was a good awareness of the importance of nutrition, and some basic information seemed clear. This could be justified by the fact that the sample of students we selected came from a school focused on education in science subjects. This suggests that to support the result of this study, a wider range of participants should be included in the future study, opening it up to schools with humanities subjects. 65% of these students had already studied the spermatozoon, and 89% recognized the correct definition of this cell. Free radicals were correctly defined by 76% of the students. 90% of them said they had already heard of antioxidants, but the correct definition was given by only 79% of them. The same situation was found for omega-3; 96% said they were already familiar with them, but 48% of the students defined them as 'proteins.' This makes one reflect on the kind of marketing that has been done in recent years in the field of food, which has seen protein described as the main macronutrient for the health and well-being of the human body, when scientifically an excess of protein is not recommended, with 0.8–1.0 g/kg/day [20] being sufficient for a healthy adult, and an excess can be deleterious in certain diseases such as chronic renal failure or liver failure. Two levels of dietary attention are presented in the game, the first showing how a diet rich in antioxidants provides the player with a reserve of munitions to scavenge ROS in seminal fluid and thus improves seminal motility.

The study demonstrates how dietary choices that prioritize omega-3 intake can help repair damage to the spermatozoa's plasma membrane. In the post-experiment questionnaire, students indicated that they had acquired specific knowledge related to their virtual reality experience. With correct answers exceeding 90% in almost all questions (except for one that was slightly more ambiguous), it can be concluded that the participants in this experiment, despite their initial good knowledge base and the necessity to broaden the study's scope, have enhanced their understanding of nutrition and reproductive biology.

The game allowed participants to expand their knowledge of diet and consolidate their previous statements. When asked what a free radical was in the pre-experiment questionnaire, the percentage of correct answers was around 70%. In the post-experiment questionnaire, the percentage increased to 85%. Students have

shown the greatest gaps in the definition and role of fatty acids in sperm health. Fatty acids are crucial for sperm health as they constitute the majority of the spermatozoa's plasma membrane, as is the case in all eukaryotic cells. This discovery suggests that the sperm cell serves as a model that can be extrapolated to all cells. The function of fatty acids is not to combat free radicals but to repair cell membranes previously damaged by them. Nonetheless, 93% of the students identified omega-3 and omega-6 PUFA as essential for sperm quality. Based on the study's findings, it can be inferred that utilizing games, particularly virtual reality, is a significant method to enhance the understanding of complex topics, ensuring the transmission of information through images and experiences endures over time. In a previous study, assessments of sickness, presence, and usability confirmed that the game was user-friendly and safe to use [21]. The study's limitations, as previously mentioned, include the small sample size of 30 students, all of whom were enrolled in a school with technical-scientific subjects. For future studies, it would be advisable to broaden the study population both quantitatively and in terms of foundational knowledge, involving institutions and high schools with diverse subject areas, to determine if virtual reality can indeed help students from non-scientific backgrounds comprehend unfamiliar topics.

Other limitations now concern the spread and cost of virtual reality; however, we must consider that the purchase of a visor in recent months has reached the cost of a normal video game, and the price will fall further, allowing students to enjoy the learning experience even at a distance. A subsequent experiment could be to compare two homogeneous groups of students who acquired knowledge in different ways: one group through traditional study (a textbook) versus another who took advantage of virtual reality. It would then be interesting, several months or a few years later, to test again using the post-experience questionnaire to see how much of what the students experienced in the virtual reality experience stayed in their memory. This assessment could be used to compare with the academic study to determine how much information lasts over time thanks to an immersive and personifying experience such as virtual reality. Combining play with learning healthy behaviors could be a new opportunity to access otherwise complex information in a simple way, to unconsciously change one's lifestyle, and to significantly prevent various diseases or disabling situations, such as infertility.

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