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Research Article

An *In-Vitro* Study to Evaluate Sperm Characteristic by Gold Nanoparticle on Healthy Fertile Men

Gholamreza Atei, Hamid Safiri, Mahboubeh Khadem Abolfazli*
Babol university medical of science, Paramedical school. Mazandaran, Iran

***Corresponding author**
Mahboubeh Khadem Abolfazli
E mail: mahboubeh.khadem@yahoo.com

Abstract: Nanoparticles differ from larger samples of the same material in their chemical and physical properties. Although human beings have been exposed to airborne nanosized particles throughout their evolutionary stages, such exposures have increased dramatically over the last century. The rapidly developing field of nanotechnology will result in new sources of this exposure, through inhalation, ingestion, skin uptake, and injection of engineered nanomaterials Published studies have shown that inhalation of nanosized materials may be harmful. This issue was studied in vitro on fertile men in Babol City with gold nano particles. The total seminal fluid from 20 healthy individual volunteer from the town Babol(Iran) that their fertility can be proved was measured gold for the content by atomic absorption wavelength 242.8nm with Hollow gold cathode lamp will be studied. Before an analysis of all collected samples on it with a mixture of citrate and thick Perchlorik to 6:1 they were analyzed. In the samples study the amount of gold in the semen of the 0.32 to 1.92 µg/ml and with a mean value 0.89 µg/ml by standard deviation 0.61µg/ml. In the present study the existing gold in the full seminal fluid after the partition was estimated. (oxidation organic materials); and have been identified as little gold levels in plasma of semen like separated sperm). So it seems that the hypothesis gold entity in sperm is right. With the available articles in this field is very low and related to previous studies therefore studies the cases in order to study on the gold role in men, is needed. With this study proved that gold in seminal fluid and decreasing the sperm movement after the influx of gold shows the forbidden of the gold for men.
Keywords: sperm, gold nanoparticles, fertile men.

INTRODUCTION

Several factors may play a role in infertility [1]. Infertility affects 13–18% of couples and growing evidence from clinical and epidemiological studies suggests an increasing incidence of male reproductive problems. The pathogenesis of male infertility can be reflected by defective spermatogenesis due to pituitary disorders, testicular cancer, germ cell aplasia, varicocele and environmental factors or to defective sperm transport due to congenital abnormalities or immunological and neurogenic factors [2]. The history may identify the causes of the infertility and detect the presence of reversible factors (drugs, smoking, endocrine diseases) [3, 4]. Semen analysis, endocrine and sperm antibody tests are useful in the identification of the aetiology of male infertility [5]. Semen analysis represents the initial test for evaluating male-factor infertility. Semen analysis includes examination of the spermatozoa and the seminal fluid, semen being amixture of spermatozoa suspended in a secretion from the testis and epididymis that iscombined with secretions from the prostate, seminal vesicles and bulbourethral glands.

Our hypothesis is probably one of the reason of male infertility is gold. So with this background the present study, we are evaluating sperm characteristic by gold nanoparticle on healthy fertile men.

MATERIALS AND METHODS

Sperm Sample

20 healthy volunteers from the town Babol and their age are between 27 to 40 years that their fertility has been proved were chosen for study. No one of these men was in the hospital for any kind of illness not hospitalized for treatment and gold cure of different forms were not received. Semen sample after these people avoid between the hours 9 to 11 that morning was collected during collection. In this process no one of volunteer should have the gold ring. Semen manual analysis based on determined standards of World Health Organization. During the initial macroscopic examination the following characteristics are considered:

1. **Liquefaction:** A normal semen sample liquefies within 60 minutes at room temperature, but usually this occurs within 15 minutes.

2. **Appearance:** A normal sample has a homogenous grey-opalescent appearance. It can be less opaque if the sperm concentration is very low and red-brown when red blood cells are present.
3. **Volume:** In normospermia the volume is 1.5–5 ml of semen/ejaculation.
4. **Viscosity:** The viscosity can be evaluated by gentle aspiration into a wide-bore 5 ml pipette. The semen is then allowed to drop by gravity. A normal sample leaves the pipette as small drops.
5. **P^H:** Semen pH is normally slightly alkaline (range 7.2–8.0).
6. **Sperm concentration:** Normozoospermic values are derived from large population studies that established a statistically significant lower conception rate when the sperm concentration was less than 20 million/ ml.
7. **Sperm motility:** The motility of spermatozoa can be classified as: Class A: rapid progressive motility. Class B: slow or sluggish progressive motility. Class C: non-progressive motility. Class D: immotility. Normal semen samples have 50% or more motile sperm, most of these exhibiting good to excellent forward progression up to 3 hours after ejaculation [6-8].

Manual analyses of the sperm of different sectors are seminal fluid separation and comparison. The motility and the quality of sperm on this basis have the triple five calibrations in the government. Level zero no motion does not show (resident). Level 1 indicates to move or motion without progress. Level 2 pointing to the sperm are moving slowly is that complex path to progress forward. Degree Q 3 indicates that the sperm move in a straight line with average speed and logical level 4 indicates the sperm move in a straight line with high speed.

The sperm are then registered in physical characteristics they were allowed to temperature of room to be turned into liquid then the number of sperm movement are then recorded and then samples are processed for more study) transferred to anyone under.

Gold Nanoparticle Solution Preparation

In the present study gold nanoparticles was produced by physical methods that invented by Sharif University. This method is called the explosion electrical wire. In general, nano particle production mechanism in this method is that the electrical voltage is very high with thin wire to enter diameter 0.1mm and caused the explosion electrical wire and change it to plasma and finally metal nanoparticle. In gold nano-colloid that it has been used in the project, the use of surfactant phosphate is before solution that has been used. The existing gold nano-colloid is 50 nm sizes with 7000 PPM or 7000 mg/ml concentration that is purchased from PNF Company.

Study of the direct in Vitro the Sperm characteristic by Gold Nanoparticle

To evaluate sperm characteristics by gold nanoparticle in the samples of semen, the samples were transferred in ice cooled box to Dr. Safiri laboratory. There seminal fluid samples was added ten ml of a mixture of nitric acid (BDH-AR) 16 N and concentrated Perchloric acid (BDH-AR) in ratio 6:1 same as Jain et al article [9]. An example for one night due to that partition is completely happen, preserved. Next day mixed in a temperature c˚ 70 for all acid to go to the remaining materials to gradually cold and 6ml of N/10 HCL will be solved. The amount of gold Levels measured by the atomic absorption spectrophotometry in standard conditions, the results measured in µg/ml. The result read in wavelength of 242.8nm. The Light source was a Cathode gold lamp and the oxidizing air used the acetylene flame (blue).

We prepared a mixture of 700 µL of gold nanoparticle solution and semen. Motility and morphological changes were studied after 15 minutes by using clinical microscopy technique under high power. As a control, motility and morphological changes of sperm were studied without the addition of the gold nanoparticle solution. The studies were performed at the Laboratory of Dr Safiri (Babolsar, Iran). During analysis samples solution at the beginning is standard and at the end route and also in period between samples analysis and or comparison to the confirmation the error of work.

RESULTS

Gold in all samples of seminal fluid analysis, played outdoors in the µg/ml 0.32 to 1.92 µ g/ml was observed for the average 0.89 µ g/ml with Standard Deviation ±0.61 is estimated was measured. The results of this research observed in the figure 1. The volume of seminal fluid has been accumulated in the amount of 2 to 4 ml and time that the liquid is played outdoors in 10 to 20 minutes. Numbers of sperm have been counted in the 96's open to 164 and the amount of sperm motility is played outdoors in 65% to 95%. In all samples white and stickiness and was zero and in the microscopic study they are not common.

According to figure 1, we can conclude with increasing gold content decreased sperm motility. Also we check sperm liquefaction time ratio gold nanoparticles observed, show in figure 2.

By attention to figure 2, we can conclude when amount of gold nanoparticle is more than 1 µ g/ml, the sperm liquefaction time is arrived highest value.

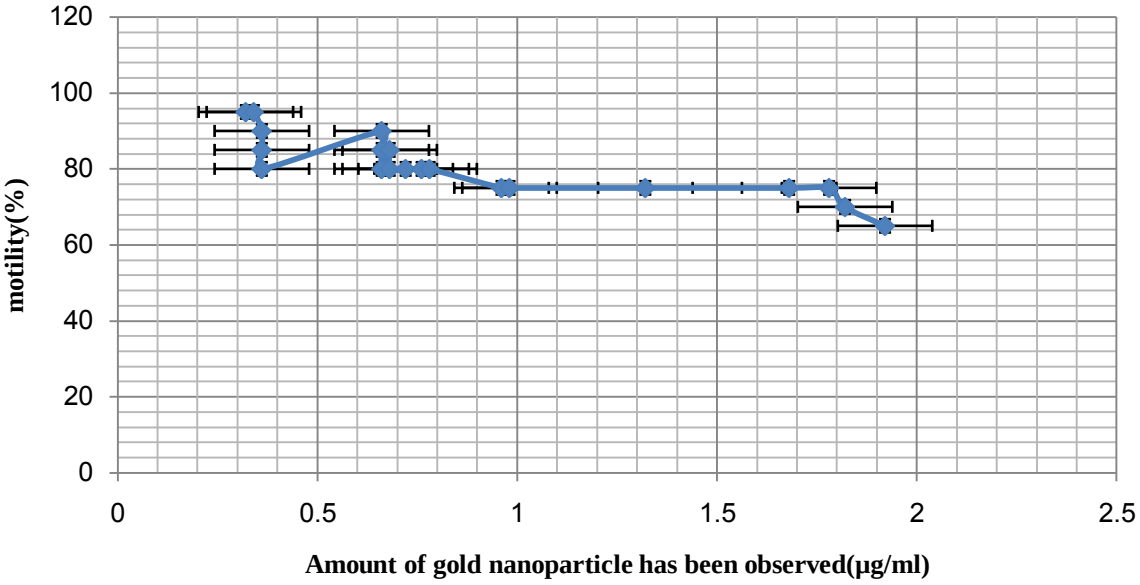

Fig-1: Show relations between sperm motility and gold observed with 5% error bar.

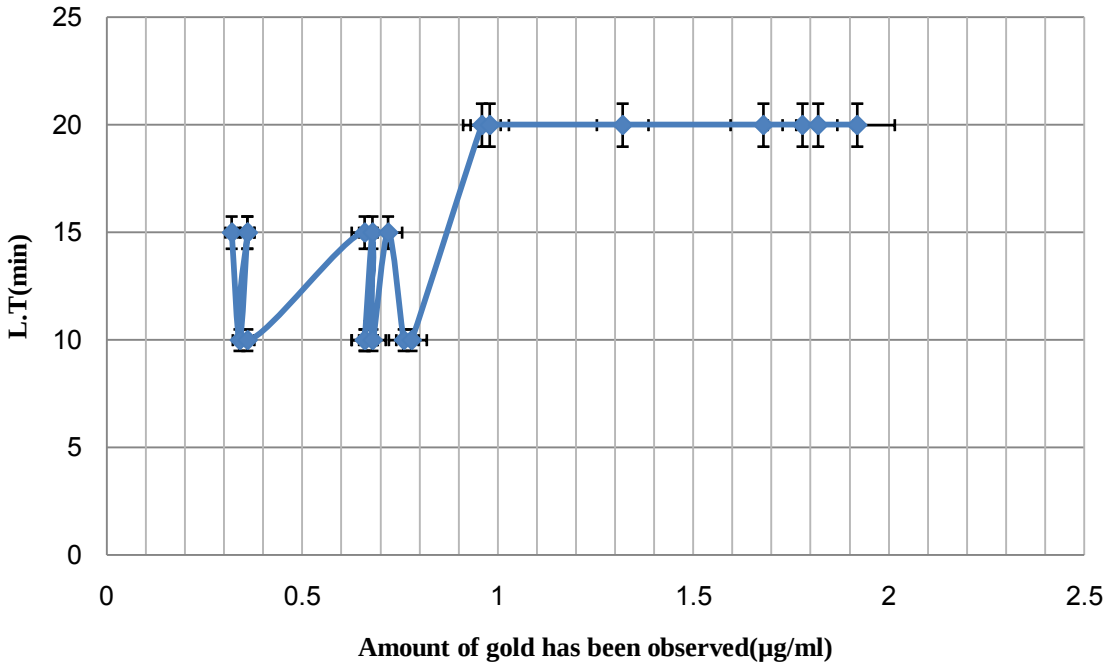

Fig-2: Show relations between sperm liquefaction time and gold observed with 5% error bar.

DISCUSSION

The effect of environmental contamination on sperm quality is well known. However, the effects of nanoparticles have been elucidated poorly. Ben-David Makhluf et al [10] performed a study to test the effect of magnetite nanoparticles and found that penetration of magnetite nanoparticles into sperm cells can be visualized. Gold nanoparticles are used widely in industry and science.

In the present study, gold is in very small particles, about 50nm, and is deposited on surfactant phosphate was estimated after complete digestion (oxidation of organic matters; hence, whatever amount of gold detected, denotes the levels in seminal plasma as well as the sperm itself) in whole semen (seminal plasma and sperm). The mean value of semen gold was found to be 0.89 µg/ml (SD ± 0.61), which is quite high when compared with the results of Skandhan [11]. Here it must be noted that Skandhan in his study had not included the sperm and did not mention about the digestion procedure (i.e. to convert all organically bound gold into inorganic forms which is the detectable form), which could be the possible cause for the high

values of gold in our study. It seems that the hypothesis made for presence of gold in sperm might be true. However, no systematic studies of gold nanoparticle in healty sperm men have been reported. We demonstrated in a preliminary, small study that the motility of healthy sperm was affected by the presence of gold nanoparticles. This differs from the case of magnetite nano- particles [10]. We also noted that gold particles can penetrate sperm cells, which could result in fragmentation [12]. But also, the literature available in this connection is very scanty and further studies are needed for scientific documentation of gold in male infertility.

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