



## Mobile Phones Reduce the Penetration Ability of Human Spermatozoa And Increase the Percentage of Swollen Sperm in the Seminal Fluid of Humans.

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**Abstract:** Generally, people hold mobile phones in the pocket of their pants, especially near the testes, and for that reason, the possible effects of mobile on reproductive health have become a matter of serious concern. The biological responses of such exposure caused various adverse effects on almost all biological systems. So, the present research has been proposed to study the biological responses of different duration of mobile phone usage on certain sperm functional parameters like penetration ability of spermatozoa and percentage of swollen sperm through an artificial medium (polyacrylamide) and Hypo Osmotic Swelling Test, respectively. For this, semen was collected from 65 men who used a mobile phone (SAR value 2.0 W/Kg averaged over 10gram tissue) at least 3 hours a week and kept mobile in their waist pocket. Semen was also collected from 22 men who never used mobile phones and were regarded as control. Their semen is analyzed according to the method given in the WHO laboratory manual for examining human semen, 5<sup>th</sup> edition, (2010). The results showed a highly significant ( $p < 0.001$ ) decreased spermatozoa penetration ability and a highly significant increased ( $p < 0.001$ ) level of percentage swollen spermatozoa in hypo-osmotic swelling test (HOST) in human subjects using mobile phones in the last 01-05 years, 06-09 years and more than 10 years of duration in comparison to non-users human subjects. Evidence from current studies suggests the potentially harmful effects of mobile phones on certain sperm functional parameters and makes the seminal fluid infertile. A further standardized study is needed to assess the risk of mobile phone use on the reproductive system.

**Keywords:** Mobile phone, Electromagnetic Radiation, Penetration ability of spermatozoa, Seminal fluid, Swollen sperm, HOST, Infertile.

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## 1. INTRODUCTION

The number of wireless subscribers was 1,148.03 million in India, and the proportion of active subscribers was approximately 88.25% at the end of July-22. The Bihar service area has a minimum teledensity<sup>1</sup> of 54.69% at the end of January-22. As the use of mobile increases, the adverse impact of it also increases. Several research articles have increased the concern about the adverse impact of the use of mobile. The various adverse effect<sup>2</sup> of mobile on humans are mainly through their thermal<sup>3</sup> and non-thermal<sup>4</sup> effects. Radiofrequency energy emitted by mobile phones is non-ionizing radiation, including electromagnetic radiation. Mobile phones emit a low level of radiofrequency in the range of 900 MHz to 2.5 GHz<sup>5</sup>. Earlier literature demonstrated that exposure to electromagnetic fields adversely affects health, particularly the reproductive system. Generally, people hold mobile phones in the front pocket, especially near the testes. For that reason, the possible effects of mobile on reproductive health have become a matter of serious concern. So, the present research has been proposed to study the impact of mobile phone usage on the penetration ability of spermatozoa and the percentage of swollen sperm in the seminal fluid of humans. Semen is a fluid produced by the male reproductive organs and composed of spermatozoa suspended in seminal fluid<sup>6</sup>. Spermatozoa are produced continuously in the seminiferous tubules of the testis and stored in the epididymis, where they undergo maturation. The spermatogenesis cycle in men takes  $74 \pm 6$  days and is controlled by anterior pituitary glycoprotein tropic hormones, namely FSH & LH, and by testicular steroid hormone testosterone. Transport of sperm through epididymis requires about 19-23 days in a man<sup>7</sup>. As the spermatozoa do not attain maturity until ejaculation, they are passively transported from the epididymis to the ampulla via vas deferens by the peristaltic movements of the latter<sup>8-10</sup>. In recent years, the conventional physical & biochemical parameters of seminal fluid are not enough determinants of

fertility in human subjects as traditional semen parameters can only provide a limited degree of prognostic and diagnostic information for assessing the fertility of an individual<sup>11</sup>. It is, therefore, necessary to develop a simple, robust, and effective test of sperm function. Recent researches consider the proteomic perspective<sup>12,13</sup> as there has been a dramatic increase in our knowledge of the protein composition of a spermatozoon, its structures, and the surrounding fluids contributing to its function. An earlier report<sup>14</sup> indicated that the measure of single sperm function traits or the result of single functional tests is poorly correlated with fertility. In this context, the investigation has been proposed to study the biological responses of different duration of mobile phone usage on certain sperm functional parameters like the Penetration ability of spermatozoa and the percentage of swollen sperm in the seminal fluid of humans.

## 2. MATERIALS AND METHODS

### 2.1. Human selection

In the present proposed investigation, all the humans of the 19-35 age group were selected based on a questionnaire containing questions related to the usage of mobile phones, like a habit of carrying the mobile, duration of use, health issues, and family history of any diseases. Men with genital issues and a family history of major diseases, Computer users, landline users, hazardous factory workers, men who lived near high radiation areas, and men who use mobile less than 3 hours a week were excluded from the present studies. The study included a total of eighty-seven young adult healthy humans (body mass index lies in-between 19 to 24). Men used mobile phones at least 3 hours a week and kept them in their waist pocket, they all were categorized into three groups (Table 1), and men who never used mobile phones were regarded as control /normal (Table 1).

**Table 1: Category of mobile phone users and non-user's human subjects (Number of humans indicated in parentheses).**

Category a	Normal, never use a mobile phone (22)
Category b	Using a mobile phone for 01-05 years of duration (25)
Category c	Using a mobile phone for 06-09 years of duration (20)
Category d	Using a mobile phone for 10 or more than 10 years of duration (20)

### 2.2. Human semen collection

The semen was collected between 2011 to 2015 from the public of Rohtas and Bhojpur districts of Bihar, India. Their semen was collected only after the public agreed to that. Semen from all categories of human subjects was collected by masturbation with a minimum of five days of abstinence. To reduce the risk of contamination, the man should pass urine,

wash his hands and penis with soap, rinse away the soap, and dry his hands and penis with a fresh disposable towel. Their semen was collected in a dry, clean, sterilized, graduated propylene, wide-mouth tube from a batch confirmed to be non-toxic for spermatozoa (WHO laboratory manual, 5th edition). It was kept for half an hour for liquefaction. Basic semen parameters are shown in Table 2.

**Table 2: Basic semen parameters**

Semen parameters	Mean value $\pm$ SD
Volume (ml)	$3.01 \pm 0.5$
Sperm density (in millions/ml)	$51.92 \pm 5.9$
Viability (in hrs.)	$5.7 \pm 1.3$
Sperm motility (in %)	$63.459 \pm 1.448$
percentage of immature DNA	$25.31 \pm 3.8$

### 2.3. Ethical statement

The study was conducted according to the guidelines of the Declaration of Helsinki for biomedical research involving human subjects. This study was approved by the Ethical and selection committee of the Veer Kunwar Singh University, Ara (letter no. (Exams.) 1881/12, dated- 26.11.2012). Written consent was taken from the patients/ individuals for participating in the study.

2.3 The penetration ability of spermatozoa was estimated through an artificial medium (polyacrylamide):

- Polyacrylamide (1.8%) was prepared according to the method of Lorton et al. (1981).<sup>15</sup>
- Micro capillaries were filled with dialyzed gel and kept at 37°C.
- 1 ml of washed spermatozoa of mobile phone users and non-users were used for penetration through Gel filled micro capillaries kept at 37°C for 90 minutes, according to the method given in the WHO laboratory manual for the examination of human semen, 5<sup>th</sup> edition, (2010).<sup>16</sup>
- Distance traveled by vanguard sperm was measured using 40X phase contrast objective lens.

### 2.4. Percentage of swollen sperm (Hypo Osmotic Swelling Test, HOST):<sup>17,18</sup>

- Preparation of swelling solution: Dissolve 0.735g sodium citrate ( $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$ ) and 1.351g fructose in 100 ml distilled water—store aliquots of this solution frozen at -20°C. Thaw and mix well before use.

- Warm 1ml swelling solution in a closed Eppendorf tube at 37°C for 5 minutes.
- Add 0.1 ml liquefied semen and mix gently with a pipette. Keep it at 37°C for at least 30 min (but not longer than 120 min) and examine the sperm cell with a phase contrast microscope.
- Swelling of sperm is identified as changes in the shape of the tail.

### 3. STATISTICAL ANALYSIS

- Data obtained of penetration ability were analyzed by student- 't-test.'
- Repeat twice the score of swollen in a total of 100 sperm counted and calculate the mean score.

### 4. RESULTS

As indicated in Table 3, a highly significant ( $p < 0.01$ ) decreased spermatozoa penetration ability was observed, and a highly significant ( $p < 0.01$ ) increased swollen spermatozoa were observed under phase contrast microscope per 100 spermatozoa at 400 X magnification in human subjects using mobile phones from 01-05 years duration and 06-09 years duration users of mobile phones in comparison to a human subject which was not using mobile phones, but this decreased spermatozoa penetration ability and increased swollen spermatozoa were highly significant ( $p < 0.001$ ) in human subjects using mobile phones since 10 years or more than 10 years in comparison to non-users human subjects.

**Table 3: Penetration of spermatozoa and Percentage of swollen sperm in mobile phone users and non-users human subjects.**

Category	Duration of mobile phone users (In years)	The penetration ability of spermatozoa (mm/15 minutes)	Percentage of swollen sperm
a	00-00	24 ± 1.57	21.31 ± 1.87
b	00-05	19 ± 1.13	27.11 ± 2.32
c	06-09	18 ± 1.49	39.31 ± 2.11
d	≥ 10	13 ± 0.76	41.21 ± 3.12

### 5. DISCUSSION

As indicated in table 3, a highly significant decreased penetration ability of spermatozoa in human subjects using mobile phones during 01-05 years, 06-09 years, and more than 10 years of duration might be an indication of low mobility of spermatozoa due to the effect of mobile phone frequency radiation. Earlier findings<sup>19,20</sup> showed that the level of reactive oxygen species production in semen was negatively co-related within the percentage of normal/mobile forms as determined by the World Health Organization<sup>21</sup> classification and Kruger's strict criteria<sup>22</sup>. When impaired spermatogenesis, disturbed spermiation gives immature and functionally defective sperms that ultimately show low penetration ability<sup>23</sup> as per our findings. An earlier report by Mailankot et al. (2009) also reported that electromagnetic field radiation generated from mobile phones in active mode disturbs free radical metabolism by enhancing reactive oxygen species<sup>24</sup> in reproductive tissue. Formation of reactive oxygen species leads to some hormonal changes<sup>25</sup>, DNA damage in spermatozoa<sup>26</sup>, and Sperm nuclear DNA fragmentation has lowered the fertilization rates in IVF<sup>27</sup>, the change in reproductive parameters, sperm functional

deterioration<sup>28</sup>, produces immature sperm during their co-migration from seminiferous tubules to the epididymis may cause infertility in men<sup>29</sup>. Oxidative stress alters redox equilibrium<sup>30</sup>, disrupts morphology and normal functioning of sperms, and declination in total antioxidant capacity. The mobile phone generates oxidative stress in rat<sup>31-33</sup> also. An alternative way of assessing sperm vitality is the Hypoosmotic swelling test (HOST), whereby spermatozoa are exposed to moderate hypo-toxic conditions. Under such conditions, dead spermatozoa, whose plasma membranes are no longer intact, do not show swelling. In addition, senescent spermatozoa with poor osmo-regularity ability show uncontrolled swelling that rapidly results in a rupture of their over-distended plasma membranes, i.e., They do not show the swelling pattern. Therefore, the proportion of spermatozoa that show controlled swelling under test conditions is considered to reflect the potentially functional fraction. This HOST test can also identify vitally but immotile spermatozoa.<sup>34</sup> As per my findings indicated in Table 3, a highly significantly increased level of percentage swollen spermatozoa in hypo-osmotic swelling test in 06-09 years and 10 years or more than 10 years of mobile phones users' human subjects might be due to the oxidative stress in

spermatozoa due to mobile phone frequency radiation. The increased level of swollen sperm after mobile phone usage adversely affects the quality of spermatozoa, which in the long run, use of mobile phone makes a man infertile. Earlier findings indicated that radiofrequency electromagnetic radiation emitted from cell phones might lead to oxidative stress<sup>35</sup> in human semen. Spermatozoa generate reactive oxygen species by NADH Oxidase system<sup>36,37</sup> at the plasma membrane level and by NADPH- dependent oxidoreductase at the level of mitochondria. Mobile radiation could induce oxidative stress and alter the protein kinase complex<sup>38</sup> of sperm. Excessive reactive oxygen species negatively change sperm quality regarding its fertilizing ability<sup>39</sup>, changes in different phases of the sperm cell cycle, and histone kinase in rats<sup>40</sup>. The radiofrequency electromagnetic radiations emitted from mobile phones enhance mitochondria<sup>41,42</sup> reactive oxygen species generation by human spermatozoa, decreasing the mobility and vitality of spermatozoa and suggesting that carrying cell phones near reproductive organs could negatively affect male fertility.

## 6. CONCLUSION

Radiofrequency radiation may harm sperm functional parameters. As for human studies, although the defined effect of mobile phone radiation on sperm functional parameters cannot be concluded from the existing studies, men should not keep mobile phones in their trouser pockets or near their testicles to avoid the potentially harmful effect of microwave radiation on the male reproductive system.

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## 8. AUTHORS CONTRIBUTIONS STATEMENT

Dr. Asit Kumar and Late Dr. Ajit Kumar Dubey designed the whole study, including sample collection, Polyacrylamide (1.8%) preparation, Preparation of swelling solution at P.G. department of zoology, H.D. Jain college (V.K.S. university, Ara). Dr. Asit Kumar prepared the manuscript. All the authors read and approved the final version of the manuscript.

## 9. CONFLICTS OF INTEREST

Conflicts of interest declared None.

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