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REVIEW ARTICLE

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Association between mobile phone use and semen quality: a systemic review and meta-analysis

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SUMMARY

Possible hazardous health effects of radiofrequency electromagnetic radiations emitted from mobile phone on the reproductive system have raised public concern in recent years. This systemic review and meta-analysis was prepared following standard procedures of the Cochrane Collaboration and the Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement and checklist. Relevant studies published up to May 2013 were identified from five major international and Chinese literature databases: Medline/PubMed, EMBASE, CNKI, the VIP database and the Cochrane Central Register of Controlled Trials in the Cochrane Library. Eighteen studies with 3947 men and 186 rats were included in the systemic review, of which 12 studies (four human studies, four in vitro studies and four animal studies) with 1533 men and 97 rats were used in the meta-analyses. Systemic review showed that results of most of the human studies and in vitro laboratory studies indicated mobile phone use or radiofrequency exposure had negative effects on the various semen parameters studied. However, meta-analysis indicated that mobile phone use had no adverse effects on semen parameters in human studies. In the in vitro studies, meta-analysis indicated that radiofrequency radiation had detrimental effect on sperm motility and viability in vitro [pooled mean difference (MDs) (95% CI): −4.11 (−8.08, −0.13), −3.82 (−7.00, −0.65) for sperm motility and viability respectively]. As for animal studies, radiofrequency exposure had harmful effects on sperm concentration and motility [pooled MDs (95% CI): −8.75 (−17.37, −0.12), −17.72 (−32.79, −2.65) for sperm concentration and motility respectively]. Evidence from current studies suggests potential harmful effects of mobile phone use on semen parameters. A further multicentred and standardized study is needed to assess the risk of mobile phone use on the reproductive system.

INTRODUCTION

'Infertility' is defined as the incapability of pregnancy after a year of sexual intercourse without the use of contraceptives (Practice Committee of the American Society of Reproductive Medicine, 2008). Infertility affects nearly 15% of couples of reproductive age, and in 50% of the cases infertility is because of the male factors (Martinez *et al.*, 2006). Our previous study on healthy men in the Chongqing area of southwest China also had indicated that semen quality was declining. The results of our investigation found that 61.1% of healthy men had at least one sperm parameter below normal threshold values compared with the World Health Organization (WHO) criteria (Li *et al.*, 2009). Both congenital and acquired factors may lead to infertility. Acquired factors include trauma, infection or exposure to toxic environmental factors (Sheiner *et al.*, 2003). The environmental factors include chemical substances, ionizing radiation, stress, as well as electromagnetic waves (Wdowiak *et al.*, 2007; Gutschi *et al.*, 2011).

Mobile phones have become an important part of everyday life (Merhi, 2012). The rapid growth of mobile phone use has been accompanied by a parallel increase in the density of electromagnetic field (EMF) (Kesari *et al.*, 2013). Public concerns have been raised regarding the potentially harmful effects of radiofrequency electromagnetic radiation (RF-EMR) emitted from mobile phones and their towers (Agarwal *et al.*, 2011). RF-EMR may have harmful effects on brain, heart, and endocrine system, and

lead to fatigue, headache, and difficulty in concentration (Agarwal *et al.*, 2011). Recent cross-sectional studies have highlighted that mobile phone use may be associated with semen quality, and it may be a growing factor contributing to male infertility (Davoudi *et al.*, 2002; Fejes *et al.*, 2005; Agarwal *et al.*, 2008). Harmful RF-EMR emitted from cell phones may interfere with normal spermatogenesis and result in a significant decrease in semen quality.

To clarify the association between mobile phone use and semen quality, many studies, including epidemiological studies, in vitro laboratory studies and animal studies have been performed to investigate this issue (Davoudi *et al.*, 2002; Fejes *et al.*, 2005; Erogul *et al.*, 2006; Wdowiak *et al.*, 2007; Yan *et al.*, 2007; Agarwal *et al.*, 2008, 2009, 2011; Gutschi *et al.*, 2011; Guan *et al.*, 2012; Veerachari & Vasan, 2012). Agarwal *et al.* (2008) reported that sperm parameters decreased with the increase use of mobile phone. Wdowiak *et al.* (2007) also reported the same results in a human study. However, Feijo *et al.* (2011) reported that sperm parameters were not significantly different in non-users and users. Therefore, it is still being debated in the literature, and a clear consensus of opinion has not emerged. With the aim of understanding the effect of mobile phone use on semen quality, we qualitatively and quantitatively reviewed all of the available literature published in English and Chinese regarding the association between mobile phone use and semen quality. We conclude with a series of recommendations regarding future intervention programmes and studies.

MATERIAL AND METHODS

Search strategy

The review was prepared following standard procedures of the Cochrane Collaboration (Cochrane Collaboration, 2008) and the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement and checklist (Moher *et al.*, 2009) (see Additional file 1). We searched Medline/PubMed (published up to May 2013), EMBASE (published up to May 2013), CNKI (China National Knowledge Infrastructure) (published up to May 2013), the VIP database (Chinese Journal of Science and Technology of VIP) (published up to May 2013) and the Cochrane Central Register of Controlled Trials (CENTRAL) in the Cochrane Library using keywords related to mobile phones and semen quality. We used a mixture of free text and index terms to maximize retrieval of potentially relevant studies. The following terms were used for the Medline/PubMed search: ('cellular phone'(MeSH Terms) OR ('cellular'(All Fields) AND 'phone'(All Fields)) OR 'cellular phone'(All Fields) OR ('mobile'(All Fields) AND 'phone'(All Fields)) OR 'mobile phone'(All Fields)) AND ('spermatozoa'(MeSH Terms) OR 'spermatozoa'(All Fields) OR 'spermatozoa'(All Fields)). The search terms used in EMBASE were phone AND ('spermatozoa'/exp OR spermatozoa). CNKI is an important national e-publishing project that can be used for searching peer-reviewed articles published in 8,200 Chinese journals. The terms and concepts searched included 'mobile phones', 'cell phones' or 'cordless phones' and 'semen', 'spermatozoa', or 'spermatozoa'. In addition, the bibliographies of retrieved reports were reviewed by hand to locate additional publications. Two reviewers (KJL and GWZ) conducted the literature searches.

Selection criteria

Inclusion/exclusion criteria

- Types of studies: Animal studies, in vitro laboratory studies and human studies (including cross-sectional studies, case–control and cohort studies) on the association between mobile phone use and semen quality were included. Only studies with a control group or comparator group were eligible for inclusion in the review.
- Participants: healthy donors and patients presenting to the infertility clinic were included in human studies and in vitro laboratory studies. Rats (including Spraque–Dawley rats, Wistar rats) and mice were used as animal models in animal studies.
- Exposure variables: frequency of mobile phone use for human studies, exposure condition including exposure devices, signal type, distance, exposure time for in vitro studies and animal studies.
- Outcomes measures: sperm concentration, motility, viability, volume and the percentage of normal morphology were mainly used to assess the semen quality.

Studies that did not provide sufficient original data to calculate the mean difference were excluded from the present meta-analysis. We have tried to contact the authors whose studies did not provide sufficient original data, but we did not receive any responses.

Selection of studies

All studies retrieved from the databases were evaluated independently by two of the authors (KJL, GWZ) based on the selection criteria. Disagreements between evaluators were resolved by discussion or in consultation with a third author (LA).

Validity assessment

We used the guideline for critical appraisal of cross-sectional studies developed by the National Collaborating Center for Environmental Health for cross-sectional studies (National Collaborating Centre for Environment Health, 2011) to assess their quality. Meanwhile, we assessed the quality of cross-sectional studies according to the method used in our previous research (Li *et al.*, 2013). The scale assesses the study based on four aspects: the representativeness of the study groups; proper methods to ascertain exposure; comparability of comparing analysis groups and lower non-response bias. We assigned a composite quality score that ranged from 0 (low) to 4 (high).

For animal studies, we assessed the quality using a gold standard publication checklist (GSPC) (Hooijmans *et al.*, 2010), which was built to improve the quality of scientific publications on animal experimentation, and to make performing systemic reviews in the animal science field more feasible. We mainly estimated the following four aspects: experiment design, comparability of experimental groups and controls, representativeness of the parameters and the rationality of the simulation devices. Meanwhile, we assigned a composite quality score that ranged from 0 (low) to 4 (high).

As for in vitro laboratory studies, there is still no standard method to assess the quality for systemic review and meta-analysis. We assess the quality of laboratory studies mainly from following four aspects: the representativeness of the participant, the rationality of the simulation devices, comparability of

experimental groups and controls and the representativeness of the outcomes, which is combined from the National Collaborating Center for Environmental Health for cross-sectional studies and GSPC. We assigned a composite quality score that ranged from 0 (low) to 4 (high) as well.

Data abstraction

All of the included studies were examined in detail. Data from relevant articles were independently abstracted by two reviewers (KJL, GWZ). Disagreement was resolved by discussion or in consultation with a third author (LA). For human studies, values of semen parameters, and the number of subjects in the exposed and not exposed to mobile phone radiation, were abstracted from each study. For in vitro studies or animal studies, values of semen parameters, and the number of semen samples or animals exposed and not exposed to radiofrequency (RF) radiation were abstracted.

Assessment of heterogeneity and data synthesis

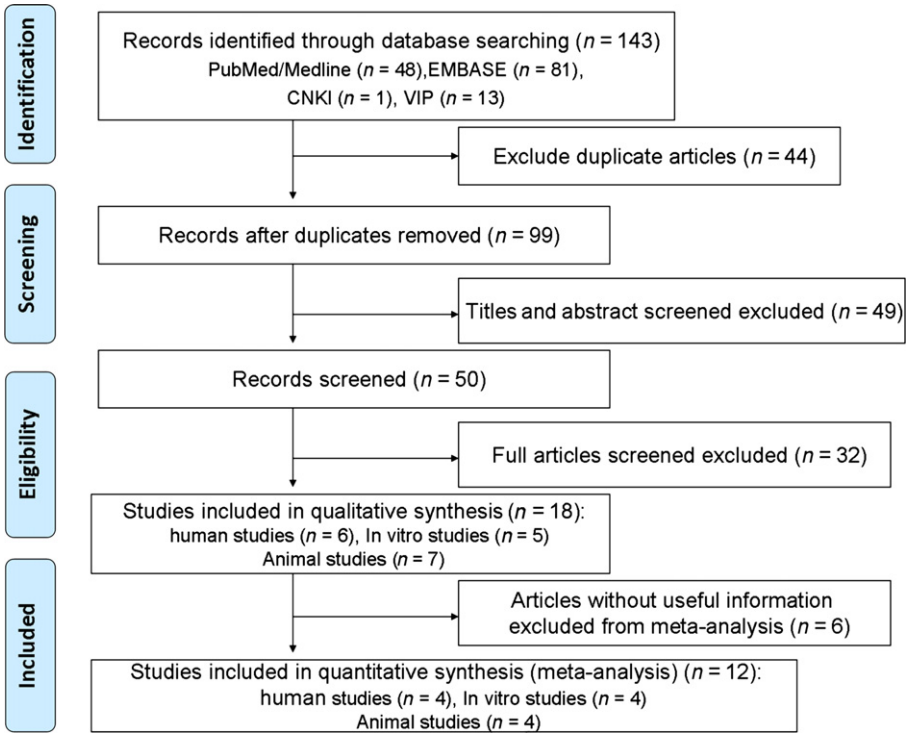
We pooled the mean differences (MD) of sperm parameters associated with mobile phone use and RF exposure by RevMan 5.2 software (Cochrane Collaboration, Oxford, UK). Before conducting the quantitative meta-analysis, we combined three or more subgroups into two groups with the method introduced in RevMan 5.2. Heterogeneity was evaluated using the Q test and the I-squared statistic. If significant heterogeneity was observed ($p < 0.10$ or $p > 0.10$ but $I^2 > 50\%$), the meta-analyses were conducted using a random effect model. A fixed effect model was used for the meta-analysis where heterogeneity was acceptable ($p > 0.10$, or $p < 0.10$ but $I^2 < 50\%$). Subgroup analyses were also performed to explore the possible reasons for the heterogeneity. In addition, sensitivity analyses were undertaken to evaluate the stability of the relationship between mobile phone use and semen quality.

RESULTS

Description of studies

A flow diagram of the review process is shown in Fig. 1. A total of 143 articles were identified with analysis on the association between mobile phone use and semen quality. Forty-four duplicate articles and an additional 87 articles were excluded because they did not meet the selection criteria. Finally, 18 articles [six human studies (Agarwal *et al.*, 2008; Feijo *et al.*, 2011; Fejes *et al.*, 2005; Gutschi *et al.*, 2011; Rago *et al.*, 2013; Wdowiak *et al.*, 2007), five in vitro studies (Erogul *et al.*, 2006; Falzone *et al.*, 2008; Agarwal *et al.*, 2009; De Iuliis *et al.*, 2009; Veerachari & Vasan, 2012) and seven animal studies (Dasdag *et al.*, 2003; Ribeiro *et al.*, 2007; Yan *et al.*, 2007; Mailankot *et al.*, 2009; Lee *et al.*, 2010; Zhang *et al.*, 2010; Guan *et al.*, 2012)] with 3947 men and 186 rats were included in the systemic review. However, only 12 articles [four human studies (Agarwal *et al.*, 2008; Feijo *et al.*, 2011; Fejes *et al.*, 2005; Rago *et al.*, 2013), four in vitro studies (Erogul *et al.*, 2006; Falzone *et al.*, 2008; Agarwal *et al.*, 2009; Veerachari & Vasan, 2012) and four animal studies (Yan *et al.*, 2007; Mailankot *et al.*, 2009; Zhang *et al.*, 2010; Guan *et al.*, 2012)] with 1533 men and 97 rats had sufficient data for inclusion in the meta-analysis. The study types of human studies were all cross-sectional studies. The samples used in the in vitro studies were all human semen from healthy donors. For animal studies, Spraque–Dawley rats, mice, Wistar rats, rabbits were used to evaluate the effect of RF radiation on semen quality. Spraque–Dawley rats and Wistar rats were widely used (Dasdag *et al.*, 2003; Ribeiro *et al.*, 2007; Yan *et al.*, 2007; Mailankot *et al.*,

Figure 1 Results of literature search. This figure is a description of the full search process.



2009; Lee *et al.*, 2010; Kesari *et al.*, 2011), so we only included the animal studies on rats, and analysed the semen parameters. Sham exposure groups were used as control groups in both animal studies and in vitro studies.

Characteristics of studies included in the final analysis

The methodological characteristics of the included studies were evaluated. The quality assessment of studies indicated that four cross-sectional studies (Fejes *et al.*, 2005; Agarwal *et al.*, 2008; Feijo *et al.*, 2011; Rago *et al.*, 2013) had a high-quality score of 4. Two studies (Wdowiak *et al.*, 2007; Gutschi *et al.*, 2011) had a score of 3, mainly because of lack of control of confounding factors. Three in vitro laboratory studies (Agarwal *et al.*, 2009; Erogul *et al.*, 2006; Veerachari *et al.*, 2012) had a quality score of 3 because the exposure devices they adopted could not provide exact exposure dosimetry. Two animal studies had a high score of 4, while one study (Guan *et al.*, 2012) had a score of 3 because of poor description of experiment design, and four studies had a score of 3 because of the poor rationality of the simulation devices (Table 1). Participants in the human studies were fertile and infertile men from infertility centres/clinics, urological centre and andrology laboratories. In three (Agarwal *et al.*, 2008; Feijo *et al.*, 2011; Rago *et al.*, 2013) of four included human studies (Fejes *et al.*, 2005; Agarwal *et al.*, 2008; Feijo *et al.*, 2011; Rago *et al.*, 2013), participants were divided into four groups according to the total hours of mobile phone use (no use, <2 h/day, 2–4 h/day and >4 h/day). We combined the original data from the four groups into two groups (no use vs. mobile phone use, <2 h/day vs. >2 h/day, <4 h/day vs. >4 h/day and no use vs. >4 h/day). The studies described clearly the inclusion/exclusion criteria and the abstinence period of participant before semen collection is 2–7 days. As for the semen quality analysis in human and in vitro studies, the included studies followed the WHO criteria. The detailed information of included articles is shown in Table 2.

Table 1 Quality assessment of the cross-sectional studies included in systematic review and meta-analysis

Studies	Study type	A	B	C	D	Total score
Agarwal *et al.* (2008)	CS	1	1	1	1	4
Feijo *et al.* (2011)	CS	1	1	1	1	4
Fejes *et al.* (2005)	CS	1	1	1	1	4
Rago *et al.* (2013)	CS	1	1	1	1	4
Wdowiak *et al.* (2007)	CS	1	1	1	0	3
Gutschi *et al.* (2011)	CS	1	1	1	0	3
Studies	Study type	A′	B′	C′	D′	Total score
Agarwal *et al.* (2009)	IVS	1	0	1	1	3
Erogul *et al.* (2006)	IVS	1	0	1	1	3
Falzone *et al.* (2008)	IVS	1	1	1	1	4
Veerachari & Vasan (2012)	IVS	1	0	1	1	3
De Iuliis *et al.* (2009)	IVS	1	1	1	1	4
Studies	Study type	A″	B″	C″	D″	Total score
Zhang *et al.* (2010)	AS	1	1	1	1	4
Guan *et al.* (2012)	AS	1	1	1	0	3
Lee *et al.* (2010)	AS	1	1	1	1	4
Ribeiro *et al.* (2007)	AS	1	0	1	1	3
Yan *et al.* (2007)	AS	1	0	1	1	3
Mailankot *et al.* (2009)	AS	1	0	1	1	3
Dasdag *et al.* (2003)	AS	1	0	1	1	3

CS, cross-sectional study; IVS, in vitro laboratory study; AS, animal study; A, representativeness of the study groups; B, proper methods to ascertain exposure; C, comparability of comparing analysis groups; D, lower non-response bias; A′, representativeness of the participant; B′, rationality of the simulation devices; C′, comparability of experimental groups and controls; D′, representativeness of the outcomes; A″, representativeness of parameters; B″, rationality of the simulation devices; C″, comparability of experimental groups and controls' D″, experiment design.

Association between mobile phone use and semen quality

Summary of systematic review

In the human studies, although results on the association between mobile phone use are fairly inconsistent (Table 2), most of the included studies (four of six) indicated that mobile phone use had negative effects on sperm parameters.

As for in vitro laboratory studies, most of studies (four in five) showed results that sperm motility and viability decreased after RF-EMR exposure (Table 2).

In animal studies, three studies (Yan *et al.*, 2007; Zhang *et al.*, 2010; Guan *et al.*, 2012) showed the results that RF-EMR exposure had harmful effects on sperm motility and viability. However, other studies did not showed the significant difference between exposure groups and control groups (Table 2).

Summary of meta-analyses

Twelve studies reported data by mean ± SD, and were used in the meta-analysis. Because the sperm parameters are continuous data, we used weighted mean difference (WMD) to estimate the effect of mobile phone use. The weight values were automatically calculated by Revman 5.2 software by inverse variance method. The reciprocal of the pooled variance was used as the weight value of each case.

In the included human studies, significant heterogeneity was observed in the sperm concentration, motility, viability and the percentage of normal morphology in the combined groups (Fig. S1, S2, S3, S4, Table 3). Only heterogeneity can be accepted in the comparison of volume ($p > 0.10$, $I^2 < 50\%$) (Fig. S1, S2, S3, S4, Table 3). However, no significant mean difference was observed in the pooled analysis (Fig. S1, S2, S3, S4, Table 3). We conducted subgroup analyses to test the source of heterogeneity according to the participant (healthy vs. infertile), comparison group (four groups vs. two groups), semen analysis criteria (WHO 4th edition vs. WHO 5th edition). However, heterogeneity still exist and the results of pooled analysis were stable (data not shown).

In the in vitro studies, where human semen samples from donors were used, heterogeneity can be accepted in the sperm motility and viability (Table 3). The fixed effects model was used for the meta-analysis. On the basis of the pooled MDs and 95% confidence intervals, it could be observed that sperm motility and viability could be influenced by RF radiation in vitro (pooled MDs (95% CI): −4.11 (−8.08, −0.13), −3.82 (−7.00, −0.65) for sperm motility and viability respectively) (Table 3, Fig. 2).

In animal studies, only the data of sperm concentration and motility were available for meta-analysis (Table 3). Although significant pooled MDs can be observed in the comparison of sperm concentration and motility (pooled MDs (95% CI): −8.75 (−17.37, −0.12), −17.72 (−32.79, −2.65) for sperm concentration and motility respectively), heterogeneity cannot be accepted ($p < 0.0005$, $I^2 = 95\%$; $p < 0.0001$, $I^2 = 92\%$,respectively) (Table 3). Subgroup analyses were performed to explore the possible reasons for the heterogeneity. Exposure condition of

Table 2 Characteristics of studies included in the meta-analysis and systemic review

Studies	Study type	Countries	Subjects	Age	Comparison group/exposure condition	Sperm parameters	Semen analysis criteria	Outcome/results
Agarwal *et al.* (2008)	Cross-sectional study	USA	361 men attending an infertility clinic	31.81 ± 6.12 years	Group A: no use (n = 40); group B:<2 h/day (n = 107); group C: 2–4 h/day (n = 100;); and group D: >4 h/day (n = 114)	Volume, liquefaction time, pH, viscosity, sperm count, motility, viability and percentage normal morphology	WHO guidelines 4th edition	Sperm parameters decreased with increased cell phones use
Feijo *et al.* (2011)	Cross-sectional study	Brazil	497 men undergoing fertility assessment	Not mentioned	Daily talk-time duration of <120 min, 120–240 min and >240 min, non-user	Sperm count, motility, viability and percentage normal morphology	WHO standard	Sperm parameters were not significantly different in non-users and users with increased cell phones use
Fejes *et al.* (2005)	Cross-sectional study	Hungary	371 men presented at clinic because of infertility problems	30.8 ± 4.4 years	Two groups: Low transmitter (<15 min/day), high transmitters (>60 min/day). Short-standby group, (<1 h/day) long-standby group (>20 h/day)	Sperm concentration, motility	WHO 4th edition	The duration of possession and the daily transmission time correlated negatively with the proportion of rapid progressive motile spermatozoa, and positively with the proportion of slow progressive motile spermatozoa
Rago *et al.* (2013)	Cross-sectional study	Italy	63 healthy and fertile men referred to centre of Andrology	18–35 years	Group A = no use (n = 10 subjects); group B = <2 h/day (n = 16 subjects); group C = 2–4 h/day (n = 17 subjects); and group D = >4 h/day (n = 20 subjects)	Volume, sperm count, motility, viability and percentage normal morphology	WHO 5th edition	None of the conventional sperm parameters examined were significantly altered. The trousers users showed a higher percentage of sperm DNA fragmentation compared to other groups.
Wdowiak *et al.* (2007)	Cross-sectional study	Poland	304 males visiting an infertility clinic	Not mentioned	Group A: 99 non-user Group B: 157 used for the period of 1–2 years, Group C: 48 used for more than 2 years	Motility	WHO 4th edition	An increase in the percentage of sperm cells of abnormal morphology is associated with the duration of exposure to GSM phone
Gutschi *et al.* (2011)	Cross-sectional study	Austria	2110 men attending an infertility clinic	31.6 ± 6.6 years	A: cell phone use (n = 991); group B: no use (n = 1119)	Sperm count, motility, morphology	WHO guidelines 3rd edition	Significant difference was observed in sperm morphology between the two groups
Agarwal *et al.* (2009)	In vitro laboratory study	USA	23 healthy donors and 9 patients presenting to the infertility clinic	Not mentioned	RF-EMR 850 MHz SAR = 1.46 W/kg. Exposed at distance of 2.5 cm for 60 min, GSM mode	Motility, viability,	WHO criteria 4th edition	A significant decrease in sperm motility and viability, increase in ROS level, and decrease in ROS-TAC score
Erogul *et al.* (2006)	In vitro laboratory study	Turkey	27 semen samples from healthy donors	27 ± 3.2 years	RF-EMR 900 MHz at distance of 10 cm for 5 min	Sperm concentration, motility	WHO criteria 4th edition	A subtle decrease in the rapid progressive and slow progressive sperm movement

Table 2 (continued)

Studies	Study type	Countries	Subjects	Age	Comparison group/exposure condition	Sperm parameters	Semen analysis criteria	Outcome/results
Falzone *et al.* (2008)	In vitro laboratory study	South Africa	12 semen samples from healthy donors	Not mentioned	Pulsed 900 MHz GSM mobile phone radiation at SAR 2.0 and 5.7 W/kg for 2 h and 24 h	Motility	WHO criteria 4th edition	No statistically significant effect of RF-EMF exposure on progressive motility of human spermatozoa
Veerachari & Vasan (2012)	In vitro laboratory study	India	20 healthy donors	Not mentioned	EMW 900 MHz, SAR = 1.46 W/kg. Exposed at distance of 2.5 cm for 60 min, GSM talk mode	Sperm count, motility, viability	WHO criteria 5th edition	A significant decrease in sperm motility and viability.
De Iuliis *et al.* (2009)	In vitro laboratory study	Australia	20 healthy donors	24.1 ± 1.1 years	RF-EMR 1.8 GHz 0.4–27.5 W/kg. Incubated for 16 h	Sperm count, motility,	Not mentioned	Motility and vitality were significantly reduced after RF-EMR exposure.
Zhang *et al.* (2010)	Animal study	China	56 Sprague–Dawley rats	Not mentioned	EMW 2856 MHz, 5 mW/cm^2, 5 min for 30 days	Sperm concentration, motility	Not mentioned	Motility was significantly reduced after RF-EMR exposure
Guan *et al.* (2012)	Animal study	China	30 Sprague-Dawley rats	Not mentioned	GSM 900 MHz, 5 mW/cm^2, 10 min for 30 days	Sperm concentration, motility, normal morphology	WHO guideline	Sperm count and motility decrease but its morphology is not related to mobile phone radiation
Lee *et al.* (2010)	Animal study	Korea	40 male Sprague–Dawley rats	30 days	CDMA 848.5 MHz RF for 12 weeks, 45-min exposure with 15-min interval, SAR = 2 W/kg	Sperm counts, frequency of spermatogenesis stages	Not mentioned	The total sperm counts in the cauda epididymis were not significantly different between the experimental and control groups
Ribeiro *et al.* (2007)	Animal study	Brazil	16 male Wistar rats	30 days	RF GSM 1,835 to 1,850 MHz) for 1 h daily during 11 weeks	Epididymal sperm count, frequency of spermatogenesis stages	Not mentioned	Epididymal sperm count was not significantly different between the groups
Yan *et al.* (2007)	Animal study	USA	16 Spraque–Dawley rat	3 months	Three hours of cell phone radiation, followed by a 30-min rest period outside of the tubes and a second exposure for three more hours per day for 18 weeks	Sperm counts	Not mentioned	Statistically significant decrease in sperm motility
Mailankot *et al.* (2009)	Animal study	India	12 Albino male Wistar rats	10–12 weeks	RF-EMR from an active GSM (0.9/1.8 GHz) mobile phone for 1 h continuously per day for 28 days	Total sperm count, motility	Not mentioned	No significant difference was observed in total sperm count between controls and RF-EMR exposed groups
Dasdag *et al.* (2003)	Animal study	Turkey	16 Spraque–Dawley rats	Not mentioned	GSM 890–915 MHz, 20 min per day for 1 month	Sperm count	Not mentioned	No significant difference was observed in total sperm count between controls and exposure groups

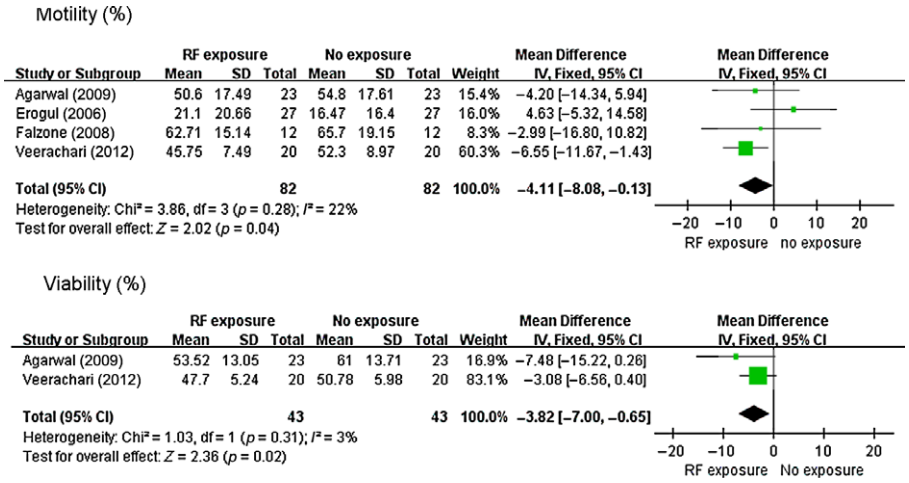
GSM, Global System for Mobile Communications; SAR, specific absorption rate; RF-EMR, radiofrequency electromagnetic radiation; RF, radiofrequency.

Table 3 Results of the meta-analysis of association between mobile phone use and semen quality

Study type	Comparison group	Sperm parameters	Number of studies	Variance between studies		Pooled mean difference		Test for overall effect (*p* value)
				Q (P)	I^2 (%)	IV	95% CI	
Human studies	Mobile phone use vs. no use	Sperm concentration	4	<0.00001	90	−1.49	(−15.85, 12.87)	0.84
		Motility	4	<0.00001	92	−3.41	(−9.49, 2.66)	0.27
		Viability	2	<0.00001	95	−4.91	(23.53, 13.72)	0.61
		Volume	2	0.47	0	0.16	(−0.30, 0.62)	0.49
		Normal morphology	3	<0.00001	97	−5.19	(−15.26, 4.88)	0.31
	Mobile phone use (>2 h/day) vs. mobile phone use (<2 h/day)	Sperm concentration	4	0.0006	80	−3.3	(−12.04, 5.44)	0.46
		Motility	4	<0.00001	96	−4.22	(−11.52, 3.09)	0.26
		Viability	2	<0.00001	99	−7.16	(−25.99, 11.68)	0.46
		Volume	2	0.96	0	0.04	(−0.27, 0.34)	0.82
		Normal morphology	3	<0.00001	98	−4.73	(−12.77, 3.30)	0.25
	Mobile phone use (>4 h/day) vs. mobile phone use (<4 h/day)	Sperm concentration	4	0.0007	79	0.73	(−9.89, 11.34)	0.89
		Motility	4	<0.00001	95	−3.6	(−10.74, 3.53)	0.32
		Viability	2	<0.00001	99	−6.61	(−27.02, 13.79)	0.53
		Volume	2	0.33	0	0.30	(−0.04, 0.64)	0.08
		Normal morphology	3	<0.00001	96	−3.80	(−10.53, 2.76)	0.26
	Mobile phone use vs. long time use (>4 h/day)	Sperm concentration	4	<0.00001	94	−1.36	(−24.20, 21.49)	0.91
		Motility	4	<0.00001	97	−6.13	(−18.18, 5.93)	0.32
		Viability	2	<0.00001	98	−8.48	(−39.61, 22.64)	0.59
		Volume	2	0.31	5	0.33	(−0.21, 0.88)	0.23
		Normal morphology	3	<0.00001	98	−7.23	(−20.94, 6.48)	0.30
In vitro studies	Exposure vs. no exposure	Motility	4	0.28	22	−4.11	(−8.08, −0.13)	**0.04**
		Viability	2	0.31	3	−3.82	(−7.00, −0.65)	**0.02**
Animal studies	Exposure vs. no exposure	Sperm concentration	4	<0.00005	95	−8.75	(−17.37, −0.12)	**0.05**
		Motility	3	<0.00001	92	−17.72	(−32.79, −2.65)	**0.02**

IV, inverse variance; 95% CI, 95% confidence interval; Q (p), Q test (probability); I^2 (%), *I*-squared statistic (%). Bold values denotes statistical significant.

Figure 2 Forest plots of meta-analysis of in vitro studies on the association between radiofrequency exposure and sperm motility and viability.

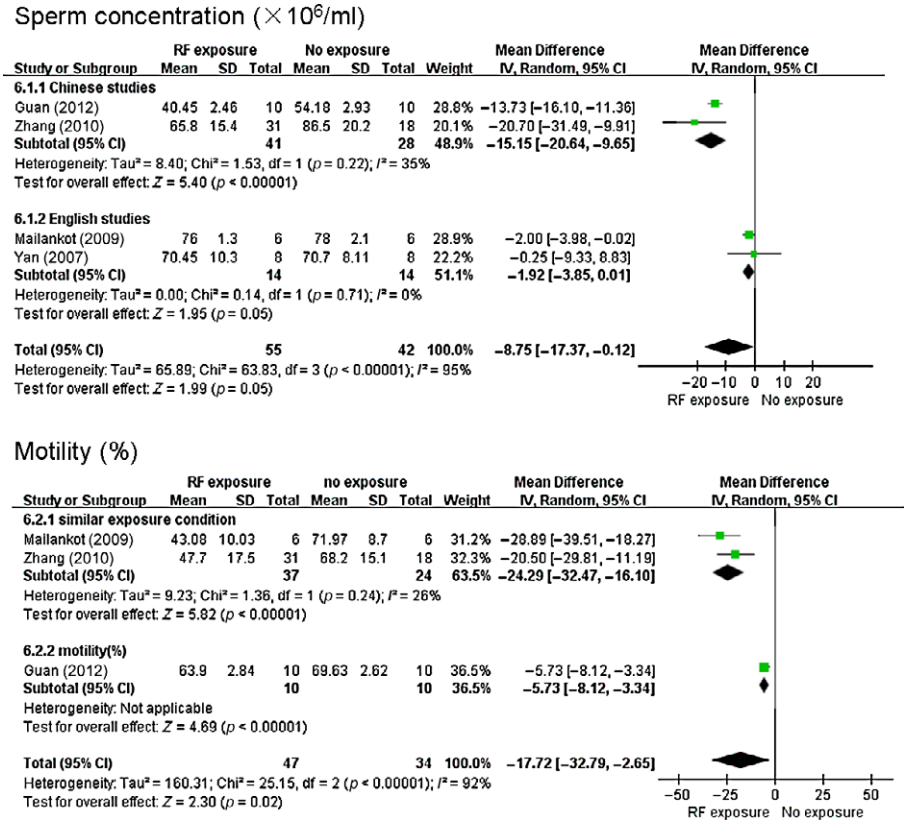


Mailankot *et al.* (2009) and Zhang *et al.* (2010) is similar, so a subgroup analysis including the two studies was conducted. No significant difference was observed in the sperm concentration (heterogeneity: $p = 0.0008$, $I^2 = 91\%$; overall effect: $p = 0.26$), but the heterogeneity can be accepted in the comparison of sperm motility ($p = 0.24$, $I^2 = 26\%$), while significant pooled MDs can be observed in the comparison of sperm motility [pooled MDs (95% CI): −24.29 (−32.47, −16.10), $p < 0.00001$] (Fig. 3). Regarding the comparison of sperm concentration, we divided the studies according to their publication languages, Chinese studies and English studies. Heterogeneity in the two subgroups can be accepted and significant mean difference was observed in two subgroups (heterogeneity: $p = 0.22$, $I^2 = 35\%$; $p = 0.71$, $I^2 = 0\%$; overall effect: $p < 0.00001$, $p = 0.05$) (Fig. 3).

Sensitivity analysis

We conducted sensitivity analysis to ascertain whether modification of the inclusion criteria of the meta-analysis influenced the final results. In the human studies, the comparison group in Fejes *et al.* (2005) is different from other studies (low transmitter vs. high transmitter). Exclusion data from Fejes *et al.* (2005) did not alter the results of heterogeneity and the overall effect. Exclusion data from Agarwal *et al.* (2008) altered the heterogeneity of the comparison of sperm concentration, motility, viability and the percentage of normal morphology in the combined groups. However, no significant pooled mean difference was observed with the exclusion of data from Agarwal *et al.* (2008).

Figure 3 Forest plots of subgroup analyses of animal studies on the association between radiofrequency exposure and sperm motility and concentration.



In in vitro studies, the study by Veerachari & Vasan (2012) plays a critical role in the results (weight: 60.3, 83.1%) because of the relatively low standard deviations (Fig. 2). After data from this study were excluded, the direction of results changed (heterogeneity: $p = 0.44$, $I^2 = 0\%$, overall effect: $p = 0.09$ for sperm motility) (Fig. S5). Thus, the results of the in vitro studies should be interpreted cautiously.

DISCUSSION

Mobile phone use has become a vital part of our life. Effects of RF emitted from mobile phone on male reproductive system have raised public concern (Agarwal *et al.*, 2011). Our study reviewed all the available published literatures that investigated the effect of mobile phone use on five sperm parameters using systematic review and meta-analysis. Systemic review showed that results of most of the human studies and in vitro laboratory studies indicate that mobile phone use or radiofrequency exposure had negative effects on semen parameters. Four human studies, four in vitro studies and four animal studies, including 1533 men and 97 rats were included in the meta-analysis.

Human studies

In human studies, based on the results of pooled analysis, mobile phone use had no definite harmful effects on semen parameters and the comparison groups and the time of mobile phone use did not affect the results. Two factors can affect the human results and may contribute to the large heterogeneity. Firstly, large variation exists in semen analysis methodologies in different laboratories. Secondly, semen parameters are not generally normally distributed, especially semen concentration. But mean values were used in the included studies, which cannot describe the actual data in populations. Compared with in vitro laboratory studies and animal studies, human studies are difficult to organize and perform, which is the reason that the amount of human studies is limited. But the results of human studies are the best evidence to clarify this issue.

In vitro studies

In the in vitro studies, the results of meta-analyses showed that RF exposure is a risk factor for sperm motility and viability. Because the experiment conditions in laboratory can be easily controlled, the confounding factors were easily excluded. Therefore, heterogeneity was acceptable in the included studies.

However, exclusion of study by Veerachari *et al.* changed the direction of the results, making the pooled results of in vitro studies instable. The quality of this study is lower than other studies because the exposure device used in this study is a commercial mobile phone (Sony Ericsson w300i), which could not provide accurate exposure level. Furthermore, authors did not mention the detailed measurement method of the SAR (specific absorption rate) value. Therefore, the results of current in vitro studies should be interpreted cautiously. Further standardized studies should be performed to provide a stable and convincing result.

Animal studies

As for the animal studies, sperm concentration and motility seems to be influenced by RF radiation exposure. Subgroup analysis showed that the possible reasons for the heterogeneity may attribute to the experiment conditions. Experiment

conditions in different laboratories are variable, so a convincing and stable result could not be concluded from the results in different laboratories. In addition, the variance of the experimental methods could explain the different results between Chinese studies and English studies.

As for Chinese and English studies, the method of sperm concentration determination is different, which may be the reason for heterogeneity of sperm concentration analysis. In Chinese studies, sperm concentration was determined immediately after rats were sacrificed. But in English studies, it was counted after the testicles were thawed or motile spermatozoa were inviable. The samples used for determining animal sperm concentration are still variable in different laboratories. Some laboratories analyse both epididymis, while some others only use one epididymis to determine the sperm concentration. A standard method to determine animal sperm concentration is needed to be set up and unified among different laboratories.

Dosimetry plays a vital role in risk evaluation of human exposure to RF fields. SAR value is the measurement for the amount of radio frequency energy absorbed by the body when using a mobile phone (Vecchia *et al.*, 2009; Agarwal *et al.*,2011). The SAR value is determined at the highest certified power level in laboratory conditions, but the actual SAR level of the mobile phone while it is operating can be well below this value (Agarwal *et al.*, 2011; Kesari *et al.*, 2013). The actual SAR level of the mobile phone is difficult to determine because the SAR distribution could be influenced by many factors, such as the type of the phone, the shape of the user's head, or the frequency (Kesari *et al.*, 2013). It is important to carefully select appropriate methods of dosimetry in each case. It is also highly recommended to validate the dosimetry by comparing with the results obtained with other methods. In the included animal and in vitro studies, seven studies (Dasdag *et al.*, 2003; Erogul *et al.*, 2006; Ribeiro *et al.*, 2007; Yan *et al.*, 2007; Agarwal *et al.*, 2009; Mailankot *et al.*, 2009; Veerachari & Vasan, 2012) adopted a commercial mobile phone as the exposure device, and provided manufacturers' SAR values without detailed measurements. Owing to the exposure device adopted in these studies, it is impossible to measure the actual SAR value. Thus, further studies should use standard exposure devices to conclude a convincing and stable result.

Agarwal *et al.* (2011) and Kesari *et al.* (2013) had reviewed the recent innovations on this topic. Their description on this topic showed similar results as our systemic review that evidence from several studies supports a growing claim that cell phone usage may have a detrimental effect on sperm parameters, leading to decreased male fertility. However, their reviews did not include systemic review and meta-analysis. In this article, we quantitatively analysed the effects of mobile phone use on sperm parameters from human studies, animal studies and in vitro laboratory studies. However, on the basis of the results of our analysis, we could not make a definite conclusion on this topic because of the conflicting results in the three kinds of studies.

The relationship between mobile phone use and alteration of sperm parameters is likely to be multifactorial, and different pathophysiological hypotheses have been raised. First, human testes need physiological temperature 2 °C lower than body temperature for optimal spermatogenesis and an elevation of testicular temperature may be reversible a detrimental factor to sperm production (Kandeel & Swerdloff, 1988; Zorgniotti, 1988; Jung & Schill, 2000). Testis depends mainly on surface conduction rather than blood flow for temperature control (Dasdag *et al.*, 1999), which can be influenced by thermal effect of RF radiation. Moreover, oxidative stress generated in the testicular organ caused by mobile phone radiation exposure leads to an increase of free radicals and reactive oxidative stress (ROS) levels in spermatozoa, which has been considered a harmful factor of male infertility (Shen *et al.*, 1999; Agarwal *et al.*, 2003; Kesari *et al.*, 2010). Both the results of human studies and animal studies showed that RF exposure can induce ROS production in spermatozoa (Falzone *et al.*, 2008; De Iuliis *et al.*, 2009; Mailankot *et al.*, 2009). Alteration of sperm cell membrane potential, apoptosis, sperm DNA damage and hormonal changes induced by mobile phone use or radiofrequency radiation contribute to the potential harmful effects of mobile phone use (Falzone *et al.*, 2008; Agarwal *et al.*, 2011). However, in the past decades, evidence for a harmful, mutagenic effect of mobile phone on male fertility is still equivocal.

Limitations

There are limitations in this study. First, bias may exist for non-published data, non-English and non-Chinese articles were not included. Second, some studies without sufficient data to calculate the mean and SD were excluded. Third, the influence of bias in this analysis could not be completely excluded because studies with positive results are easier to publish. Fourth, the number of current studies is limited. Moreover, although we conducted a subgroup analysis, there is still heterogeneity between different studies. Recall bias often exist in human studies because questionnaire was used to assess the frequency of mobile phone use in these studies. The participants included in human studies are primarily the men attending an infertility clinic, so that the sample is highly biased. We could not exclude these kinds of bias because of the studies themselves. Meanwhile, because the studies were not randomized, potential confounders of the relationship between exposure and outcome exist, but it is impossible for us to control in the meta-analysis. We would like to assess the association between mobile phone use and infertility, but pooled odds ratio (ORs) cannot be calculated because of lack of original data and studies.

Implications for practice and research

The review showed that heterogeneity was significant in the human studies. The heterogeneity may originate from study design, recall bias in each study, exposure system. Therefore, a multicentred and standardized study for the association between mobile phone use and semen quality in human population is needed to assess the risk of mobile phone use on reproductive system, like the 'Interphone' study to assess the risk of mobile phone use on brain cancer. From the subgroup analysis of animal studies, publication language and exposure condition were all sources of heterogeneity. As for in vitro studies and animal studies, exposure conditions, including exposure devices, signal types and exposure times, should be standardized to assess the results from different laboratories. Therefore, current debate on effect of RF has to be explored through proper guidelines for exposure system and also their bio-interaction mechanism as well as measurement of exposure parameters. Meanwhile, it is necessary to find out the biomedical applications to protect RF-EMF emitted from mobile phone use.

CONCLUSION

Radiofrequency radiation may have a harmful effect on human semen quality in vitro, and in animal studies. As for human studies, although the defined effect of mobile phone use on semen quality cannot be concluded from the existing studies, men should not keep mobile phone in their trousers pockets or near testicles to avoid the potential harmful effect of radiofrequency radiation on the male reproductive system. Further well-designed and standardized case–control and cohort studies are needed to identify the effect of mobile phone use on semen quality and the association between mobile phone use and infertility.

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CONFLICT OF INTEREST

None.

AUTHOR CONTRIBUTION

S.X.Z. and L.A. designed the study. K.J.L. and G.W.Z. designed the search strategies and searched the literatures. K.J.L., J.Y.L., J.C. and L.A. analysed the data. K.J.L. and Y.L. drafted the manuscript. K.J.L. and G.W.Z. selected the studies and abstracted the data. L.A., S.X.Z. and Y.L. edited the manuscript. All authors read and approved the final manuscript.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1. Forest plots of meta-analysis of human studies on the association between mobile phone use and no use.

Figure S2. Forest plots of meta-analysis of human studies on the association between mobile phone use (>2 h/day) and mobile phone use (<2 h/day).

Figure S3. Forest plots of meta-analysis of human studies on the association between mobile phone use (>4 h/day) and mobile phone use (<4 h/day).

Figure S4. Forest plots of meta-analysis of human studies on the association between mobile phone use and long time use (>4 h/day).

Figure S5. Forest plots of meta-analysis of in vitro studies after study by Veerachari 2012 was excluded.