EPIDEMIOLOGY OF DRINKING WATER FLUORIDE AND ITS CONTRIBUTION TO FERTILITY, INFERTILITY, AND ABORTION: AN ECOLOGICAL STUDY IN WEST AZERBAIJAN PROVINCE, POLDASHT COUNTY, IRAN

Mahmood Yousefi,[a] Ali Akbar Mohammadi,[b] Mehdi Yaseri,[c] Amir Hossein Mahvi [a,d,*]

Tehran and Neyshabur, Iran

ABSTRACT: Research, including animal studies, shows that the fluoride ion (F) adversely impacts the reproductive system in mammals. However, the gene-environment interaction mechanisms involved in this association remain unclear. The objective of the present study was to measure the F level in two regions of Poldasht county, Iran, with low and high drinking water F levels (means 1.90 and 8.10 mg/L, respectively) and to examine, in females living in the areas, the relationship between the drinking water F and (i) fertility, (ii) infertility without known etiological factors, and (iii) abortion without known etiological factors. Comparing the women in the low and high F areas, the respective rates were: (i) fertility: 105/1993, 5.3%; 70/1224, 0.5%; (ii) infertility without known etiological factors: 17/1993, 0.9%; 24/1224, 2.0%; and (iii) abortion without known etiological factors: 6/105, 5.7%; 11/70, 10.0%. We found that there were no statistically significant differences in the reproductive parameters between the low and high F regions when the women were considered by 5-year age groups, but, when the data were pooled and all the age groups were considered together in a group with ages 10–49 yr, those in the low F group were more fertile ($p<0.05$) and had lower rates of (i) infertility without known etiological factors ($p<0.001$) and (ii) abortion without known etiological factors ($p<0.001$).

Keywords: Abortion; Drinking water; Fertility; Fluoride; Infertility; Iran; Poldasht.

INTRODUCTION

The fluoride ion (F) at high concentrations in drinking water has a negative effect on human life and a guideline value for the upper limit of F in drinking water of 1.5 mg/L was set by the WHO in 1984 and reaffirmed in 1993.[1] However, in setting national standards or local guidelines for F, or in evaluating the possible health consequences of exposure to F, it was noted that it was essential to consider the total intake in the population of interest from both water and other sources such as food and air.[1] Where the intakes were likely to approach or be greater than 6 mg/day, a guideline concentration for the upper limit of F in drinking water of >1.5 mg/L was seen to be appropriate.[1]

A dental fluorosis prevalence of 12–33% has been reported with drinking water concentrations of 0.9–1.2 mg/L and a high F intake may cause serious toxic effects in consumers such as anemia in pregnancy, low birth weight babies, neurological problems and lowered IQ, chronic cognitive impairment and dementia, disturbed thyroid hormone metabolism, and infertility problems.[2-11] The adverse effects of F on human health may be treatable, or permanent and irreversible.[12] The World

[a]Department of Environmental Health Engineering, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran; [b]Department of Environmental Health Engineering, Neyshabur University of Medical Sciences, Neyshabur, Iran; [c]Department of Epidemiology and Biostatistics, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran: [d]Center for Solid Waste Research, Institute for Environmental Research, Tehran University of Medical Sciences, Tehran, Iran; For correspondence: Assistant Professor Amir Hossein Mahvi, Center for Solid Waste Research, Institute for Environmental Research, Tehran University of Medical Science, Tehran, Iran; E-mail: ahmahvi@yahoo.com

Health Organization defines the term infertility as a failure to achieve a successful pregnancy after 12 months or more of appropriate, timed, unprotected sexual intercourse. About 10% to 15% of all couples are unable to conceive a child after 1 year of trying to become pregnant.[13] Many factors such as environmental and lifestyle factors can affect human fertility.[14-16] Freni reported in 1994 that a high content of F in drinking water could lead to a decrease in the rate of reproduction.[17] A variety of clinical studies have found a correlation between the long-term uptake of drinking water with a high concentration F and impaired reproduction. Research has shown that high F exposure is associated with decreased estrogen and testosterone levels. In addition, the adverse results found include interference with spermatogenesis, a decline in sperm motility, and a decrease in the sperm count and other reproductive functions.[17-22]

Infertility is one of the most fundamental problems in the world, and is a growing global public health issue. In the Republic of Iran, 2 in 10 couples (20%) will experience difficulty in conceiving. Comparable societies report rates between 8% and 20%.[23-24] The annual incidence of infertility is estimated at 200 couples per 1000 total general population. In Iran's population of 70 million, 3 million couples are grappling with infertility.[25] Infertility is a complex disorder and can be caused by multiple factors including physical, chemical, biological, behavioral, social, and economic. The environmental factors that may affect reproductive health include exposure, during critical periods of human development, to risk factors such as contamination of drinking water with lead, arsenic, chromium, and F.[26-28] The effects of F on fertility and infertility, in both women and men, is a matter of concern in the scientific community. The role of F on reproductive health system is complex and the mechanisms involved are uncertain. Although many studies have been carried out world-wide on F and fertility, few have been epidemiological studies on the correlation between human F exposure and the prevalence of fertility, infertility, and abortion.

Thus, the objectives of the present study were to examine the relationship between the prevalence of fertility, infertility, and abortion in females who were exposed to high and low levels of F in drinking water in West Azerbaijan Province, Poldasht County, Iran.

MATERIAL AND METHODS

Study areas: Poldasht County is located in West Azerbaijan Province in north western Iran with UTM coordinates: zone/sector 39S, 446625–513055 m E, and 4344280–4402863 m N. The Poldasht meteorological station showed that in the long-term, the average rainfall was equal to 131.5 mm.[29] The County has a border in the west and north with Turkey. Two study areas (five villages) were selected in Poldasht County with almost the same socioeconomic status and dietary habits but different natural concentrations of F in their drinking water. Two villages had a high level of F (Sarisoo and Konikor) and three villages had a low level of F (Daiankendi, Eshgabad, and Moradloo).

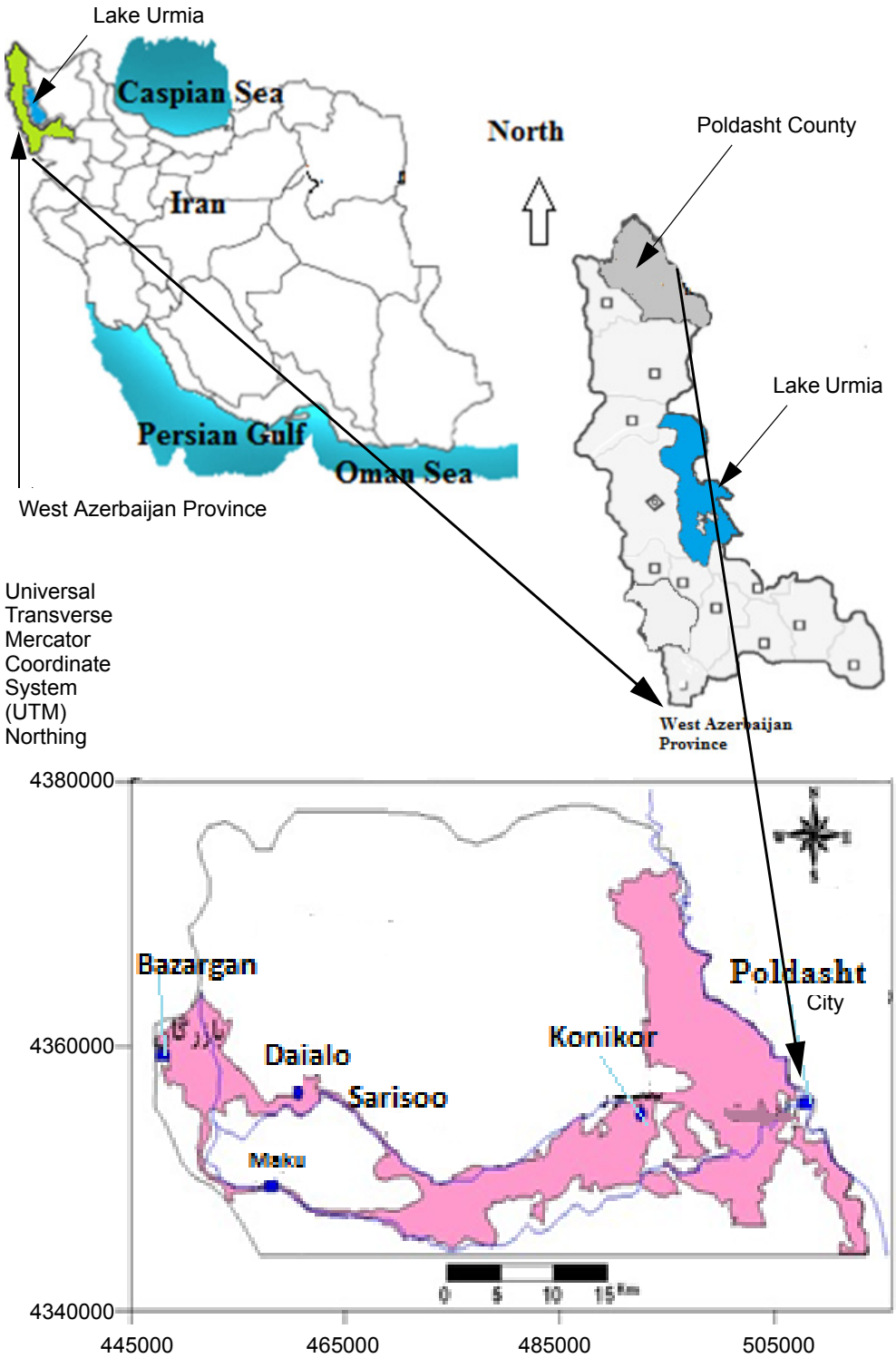

Figure 1. Location of study sites.

Determination of the water fluoride concentration: Five drinking water wells in the area were selected. A total of 20 samples were collected over three consecutive years in 2013, 2014, and 2015. The water samples were collected from the ground water wells in sterile plastic 2 L containers and then transported to the Laboratory for Water and Sewage Poldasht. The F concentration in the water samples was determined using the SPADNS method according to the standard method. [30]

Data gathering for fertility: The data on the prevalence of fertility, infertility, and abortion, were derived from the health records of the areas being investigated. Based on the health records, 2098 women in the high F area (Sarisoo and Konikor) and 1294 people in the low F area (Daiankendi, Eshgabad, and Moradloo) were identified. Many women were excluded from the study because of the presence of known causes of infertility such as diabetes mellitus, obesity, smoking, and consumption of alcohol.

Statistical analysis: To present the data we used the mean standard deviation, frequency, and percent. To compare the groups regarding the rate and prevalence of the outcomes, we used Poisson regression and logistic regression analysis within a GEE method to consider the possible correlation of area with time. All statistical analyses were performed with the SPSS (IBM SPSS Statistics for Windows. Version 24.0. released 2016. Armonk, NY: IBM Corp; 2016.) p-values less than 0.05 were considered to be statistically significant.

RESULTS

All the subjects in the study were residents of Poldasht County, an endemic fluorosis area. In the rural areas studied, the range of F concentrations in the drinking water was 1.46–10.3 mg/L. Eighty-five % of the samples showed a F level higher than the WHO recommended upper limit of 1.5 mg/L and 15% of the water samples were below this in the acceptable range of <1.5 ppm (Table1).

Table 1. Drinking water fluoride (F) concentration (mg/L) in 2013–2015 in the rural areas studied of Poldasht County, Iran

Drinking water F parameter	Drinking water F concentration (mg/L)	
	Low F villages (Daiankendi, Eshgabad, Moradloo)	High F villages (Sarisoo, Monikor)
Mean	1.90	8.10
Standard deviation	0.37	1.44
Median	1.76	8.28
Minimum	1.46	6.00
Maximum	2.81	10.30

The results showed that the mean F concentration in the groundwater resources in the five villages studied was higher than the maximum guideline level of the WHO of 1.5 mg/L. The lowest mean concentration of F was 1.46±0.37 mg/L in Moradloo village and highest mean F level was 10.3±1.44 mg/L in Sarisoo village (Table 1). Comparing the women in the low and high F areas, the respective fertility, infertility, and abortion rates were: fertility: 105/1993, 5.3%; 70/1224, 0.5%; infertility without known etiological factors: 17/1993, 0.9%; 24/1224, 2.0%; and abortion without known etiological factors: 6/105, 5.7%; 11/70, 10.0%. (Tables 2–5). We found that there were no statistically significant differences in the reproductive parameters between the low and high F regions when the women were considered by 5-year age groups, but, when the data were pooled and all the age groups were considered together in a group with ages 10–49 yr, those in the low F group were more fertile ($p<0.05$) and had lower rates of (i) infertility without known etiological factors ($p<0.001$) and (ii) abortion without known etiological factors ($p<0.001$).

Table 2. Comparison of the prevalence of fertility (%) in the different age groups in the rural areas with different drinking water fluoride levels

Age group (yr)	Group						Difference	p
	Low F villages (Daiankendi, Eshgabad, Moradloo)			High F villages (Sarisoo, Monikor)				
	Population	n	Prevalence of abortion (%)	Population	n	Prevalence of abortion (%)		
10–14	293	0	0.0%	225	0	0.0%	0.0%	1
15–19	308	14	4.5%	247	11	4.5%	–0.1%	0.843
20–24	347	29	8.4%	199	22	11.1%	2.7%	0.62
25–29	274	30	10.9%	134	25	18.7%	7.7%	0.089
30–34	255	19	7.5%	108	10	9.3%	1.8%	0.73
35–39	178	10	5.6%	108	2	1.9%	–3.8%	0.152
40–44	199	3	1.5%	93	0	0.0%	–1.5%	0.998
45–49	139	0	0.0%	110	0	0.0%	0.0%	1
Total	1993	105	5.3%	1224	70	5.7%	0.5%	<0.001

Table 3. Comparison of the prevalence of infertility (%), without known etiological factors, in the different age groups in the rural areas with different drinking water fluoride levels

Age group (yr)	Group						Difference	p
	Low F villages (Daiankendi, Eshgabad, Moradloo)			High F villages (Sarisoo, Monikor)				
	Population	n	Prevalence of abortion (%)	Population	n	Prevalence of abortion (%)		
10–14	293	0	0.0%	225	0	0.0%	0.0%	1
15–19	308	0	0.0%	247	0	0.0%	0.0%	1
20–24	347	0	0.0%	199	2	1.0%	1.0%	0.132
25–29	274	2	0.7%	134	11	8.2%	7.5%	0.018
30–34	255	2	0.8%	108	7	6.5%	5.7%	0.002
35–39	178	9	5.1%	108	4	3.7%	–1.4%	0.342
40–44	199	3	1.5%	93	0	0.0%	–1.5%	–
45–49	139	1	0.7%	110	0	0.0%	–0.7%	–
Total	1993	17	0.9%	1224	24	2.0%	1.1%	<0.001

Table 4. Comparison of the prevalence of abortion (%), without known etiological factors, in the different age groups in the rural areas with different drinking water fluoride levels

Age group (yr)	Group						Difference	p
	Low F villages (Daiankendi, Eshgabad, Moradloo)			High F villages (Sarisoo, Monikor)				
	Population	n	Prevalence of abortion (%)	Population	n	Prevalence of abortion (%)		
10–14	0	0	NA	0	0	NA	NA	–
15–19	14	0	0.0%	11	2	18.2%	18.2%	0.183
20–24	29	2	6.9%	22	2	9.1%	2.2%	0.922
25–29	30	1	3.3%	25	4	16.0%	12.7%	0.007
30–34	19	2	10.5%	10	2	20.0%	9.5%	0.257
35–39	10	1	10.0%	2	1	50.0%	40.0%	0.336
40–44	3	0	0.0%	0	0	NA	NA	–
45–49	0	0	NA	0	0	NA	NA	–
Total	105	6	5.7%	70	11	15.7%	10.0%	0.011

DISCUSSION

Some of the F concentration values of the water resources in Poldasht County are higher than the recommended WHO upper limit guideline of 1.5 mg/L. The lowest mean concentration of F was 1.46±0.37 mg/L in Moradloo village and the mean highest F level was 10.3±.1.44 mg/L in Sarisoo village (Table 1). Various adverse effects on human tissue of high drinking water F concentrations have been observed[31-32] but the adverse effects on the reproductive system are still not well understood. The results of the present study showed that there was no statistically significant difference between the low and high F villages when the women were considered by 5-year age groups, but, when the data were pooled and all the age groups were considered together in a group with ages 10–49 yr, those in the low F group were more

fertile ($p<0.05$), and had lower rates of (i) infertility without known etiological factors ($p<0.001$) and (ii) abortion without known etiological factors ($p<0.001$).

A similar epidemiological study by Freni et al. in 1994 in the United States of America examined the effect of F in drinking water on the reproduction rate in women and found that an increased F intake was associated with a reduced reproduction rate.[17]

Another study, by Ortiz-Perez et al. in 2003 in Mexico, compared people working at a factory producing aluminum fluoride and hydrofluoric acid that were exposed to F at high doses of 3–27 mg/day to a control group exposed to lower doses of F of 2–13 mg/day.[33] In the high F exposure group there was (i) a significant increase in FSH ($p<0.05$); (ii) a reduction of inhibin-B, free testosterone, and prolactin in serum ($p<0.05$); and (iii) a decreased sensitivity in the FSH response to inhibin-B ($p<0.05$). In addition there was (a) a significant negative partial correlation between urinary F and serum levels of inhibin-B ($r= –0.333$, $p=0.028$) in the low F exposure group; and (b). a significant partial correlation between a chronic exposure index for F and the serum concentrations of inhibin-B ($r= –0.163$, $p=0.037$) in the high F exposure group.[33] The authors considered that their results indicated that a F exposure of 3–27 mg/day induced a subclinical reproductive effect that could be explained by a F-induced toxic effect in both Sertoli cells and gonadotrophs.[33]

A further study, by Al-Hiyasat and Elbetieha in 2000, conducted on female Sprague-Dawley rats, used F concentrations in drinking water of 0.5, 200. 400, and 600 mg/L.[34] The results showed that high concentrations of F had an adverse effect on the reproductive system of the rats with more resorptions and a fetotoxic effect with fewer viable fetuses.[34]

Other studies in this area on human society have suggested that high concentrations of F cause changes in the reproductive hormones and increase the risk of Down syndrome but these investigations have their limitations.

A case-control study by Diouf et al. in 2012 in Senegal on pregnant women living in a fluorosis endemic area with high water F levels included 108 mothers who gave birth to newborns weighing less than 2500 g (cases) and 216 mothers with newborns weighing greater or equal to 2500 g (controls).[35] The proportions of mothers consuming well water, with a higher F concentration, were 62% among the cases versus 43.5% among the controls.[35] A score 4 on Dean's Index was reported for 25.9% of the cases versus 6.9% of the controls.[35] The water consumed and the modal score on Dean's Index were significantly associated with the occurrence of low birthweight adjusted for gender, consanguinity, anemia, and hypertension.[35] The authors concluded that low birthweight was associated with pregnant women living in endemic areas. and that defluoridation programs and access for pregnant women and children to high quality water were necessary in areas of endemic fluorosis.[35]

An additional clinical study, by Susheela in 2010, was focused on the relationships between drinking water F concentration, premature birth, and anemia.[36] The results showed that F lowered the production of red blood cells and eventually caused premature death of red blood cells.[36]

In general, the existing studies on the relationship between the F content in drinking water and human reproduction are few in number and they have significant deficiencies in their design and power, thus restricting the ability to draw conclusions from them.

CONCLUSIONS

The present study indicates that the groundwater of Poldasht is highly deteriorated due to pollution with the fluoride ion. Eighty-five % of the samples in the five villages studied had a F level higher than the WHO recommended upper limit guideline for drinking water F of 1.5 mg/L. We found that there were no statistically significant differences in the reproductive parameters between the low and high F regions when the women were considered by 5-year age groups, but, when the data were pooled and all the age groups were considered together in a group with ages 10–49 yr, those in the low F group were more fertile ($p<0.05$) and had lower rates of (i) infertility without known etiological factors ($p<0.001$) and (ii) abortion without known etiological factors ($p<0.001$). Finally, the authors declare that ultimate conclusions about the association of fluoride and fertility, infertility without known aetiological factors, abortion without known aetiological factors, and other effects on reproductive health will require more experimental and epidemiologic studies, including a better control of confounding factors.

ACKNOWLEDGEMENTS

The authors want to thank the authorities of the Poldasht Health Center for their contributions in assisting the collection of information.

REFERENCES

1 WHO. Fluoride in drinking-water: background document for development of WHO Guidelines for drinking-water quality. WHO/SDE/WSH/03.04.96, English only. Geneva: WHO; 2004. Available from: http://www.who.int/water_sanitation_health/dwq/chemicals/fluoride.pdf

2 Dobaradaran S, Abadi DRV, Mahvi AH, Javid A. Fluoride in skin and muscle of two commercial species of fish harvested off the Bushehr shores of the Persian Gulf. Fluoride 2011;44(3):143-6.

3 Karimzade S, Aghaei M, Mahvi AH. Investigation of intelligence quotient in 9–12-year-old children exposed to high and low-drinking water fluoride in West Azerbaijan Province, Iran. Fluoride 2014;47(1):9-14.

4 Dobaradaran S, Fazelinia, Mahvi AH, Hosseini SS. Particulate airborne fluoride from an aluminium production plant in Arak, Iran. Fluoride 2009;42(3):228-32.

5 Aghaei M, Karimzade S, Yaseri M, Khorsandi H, Zolfi E, Mahvi AH. Hypertension and fluoride in drinking water: case study from West Azerbaijan, iran. Fluoride 2015;48(3):252-8.

6 Mahvi AH, Zazoli MA, Younecian M, Nicpour B, Babapour A. Survey of fluoride concentration in drinking water sources and prevalence of DMFT in the 12 years old students in Behshar City. Journal of Medical Sciences 2006;6(4):658-61.
7 Amouei AI, Mahvi AH, Mohammadi AA, Asgharnia HA, Fallah SH, Khafajeh AA. Fluoride concentration in potable groundwater in rural areas of Khaf city, Razavi Khorasan Province, Northeastern Iran. Int J Occup Environ Med 2012;3(4):201-3.
8 Rhmani A, Rahmani K, Dobaradaran S, Mahvi AH, Mohamadjani R, Rahmani H. Child dental caries in relation to fluoride and some inorganic constituents in drinking water in Arsanjan, Iran. Fluoride 2010;43(4):79-86.
9 Faraji H, Mohammadi A.A, Akbari-Adergani B, Saatloo N.V, Lashkarboloki G, Mahvi A.H. Correlation between fluoride in drinking water and its levels in breast milk in Golestan Province, Northern Iran. Iran J Public Health 2014;43:1664.
10 Asghari FB, Mohammadi AA, Aboosaedi Z, Yaseri M, Yousefi M. Data on fluoride concentration levels in cold and warm season in rural area of Shout (West Azerbaijan, Iran). Data in Brief 2017;15:312-5.
11 Mohammadi AA, Yaghmaeian K, Hossein F, Nabizadeh R, Dehghani MH, Khaili JK, Mahvi AH. Temporal and spatial variation of chemical parameter concentration in drinking water resources of Bandar-e Gaz City using Geographic Information System. Desalination and Water Treatment 2017;68:170-6.
12 Sharma JD, Solanki M, Solanki D. Sodium fluoride toxicity on reproductive organs of female albino rats. Asian J Exp Sci 2007;21(2):359-64.
13 Oliva A, Spira A, Multigner L. Contribution of environmental factors to the risk of male infertility. Human Reproduction 2001;16(8):1768-76.
14 Negro-Vilar A. Stress and other environmental factors affecting fertility in men and women: overview. Environmental Health Perspectives 1993;101(2):59-64.
15 Younglai EV, Holloway AC, Foster WG. Environmental and occupational factors affecting fertility and IVF success. Human Reproduction Update 2005;11(1):43-57.
16 Long H, Jin Y, Lin M, Sun Y, Zhang L, Clinch C. Fluoride toxicity in the male reproductive system [review]. Fluoride 2000;42(4):260-76.
17 Freni SC. Exposure to high fluoride concentrations in drinking water is associated with decreased birth rates. J Toxicol Environ Health 1994;42:109-12.
18 Irvine DS. Epidemiology and etiology of male infertility. Human Reproduction 1998;13 Suppl 1:33-44.
19 Spittle B. Halting the inertia of indifference: fluoride and fertility revisited [editorial]. Fluoride 2009;42(3):159-61.
20 Chinoy NJ, Narayana MV. *In vitro* toxicity in human spermatozoa. Reproductive Toxicology 1994;8:155-9
21 Veeramachaneni DN. Impact of environmental pollutants on the male: effects on germ cell differentiation. Anim Reprod Sci 2008;105(1-2):144-57.
22 Clementi M, Tiboni GM, Causin R, La Rocca C, Maranghi F, Raffagnato F, Tenconi R. Pesticides and fertility: an epidemiological study in Northeast Italy and review of the literature. Reprod Toxicol 2008;26(1):13-8.
23 Kour K, Singh J. Histological finding of mice testes following fluoride ingestion. Fluoride 1980;13:160-2.

24 Case AM. Infertility evaluation and management: strategies for family physicians. Can Fam Physician 2003;49:1465-72.

25 Rostami Dovom M, Ramezani Tehrani F, Abedini M, Amirshekari G, Hashemi S, Noroozzadeh M. A population-based study on infertility and its influencing factors in four selected provinces in Iran (2008-2010). Iran J Reprod Med 2014;12(8):561-6.

26 Elbetieha A, Darmani H, Al-Hiyasat AS, Fertility effects of sodium fluoride in male mice, Fluoride 2000;33(3):128-34

27 Birnbaum L. The effects of environmental chemicals on human health. Fertility and Sterility 2008;89(1):e31.

28 Sharma R, Biedenharn KR, Fedor J M, Agarwal A. Life style factors and reproductive health: taking control of your fertility. Reproductive Biology and Endocrinology 2013;11(66):1-15.

29 Mohammadi AA, Yousefi M, Mahvi AH. Fluoride concentration level in rural area Poldasht city and daily fluoride intake based on drinking water consumption with temperature. Data in Brief 2017;13:312-5.

30 American Public Health Association, Eaton AD, Water Environment Federation, American Water Works Association. Standard methods for the examination of water and wastewater. 21st ed. Washington, DC, USA: prepared and published jointly by American Public Health Association, American Water Works Association and Water Environment Federation; 2005.

31 Liu H, Gao Y, Sun L, Li M, Li B, Sun D. Assessment of relationship on excess fluoride intake from drinking water and carotid atherosclerosis development in adults in fluoride endemic areas, China. International Journal of Hygiene and Environmental Health 2014:217(2-3);413-20.

32 Sun L, Gao Y, Liu H, Zhang W, Ding Y, Li B, et al. An assessment of the relationship between excess fluoride intake from drinking water and essential hypertension in adults residing in fluoride endemic areas. Sci Total Environ 2013;443:864-9.

33 Ortiz-Pérez D, Rodríguez-Martínez M, Martínez F, Borja-Aburto VH, Castelo J, Grimaldo JI, et al. Fluoride-induced disruption of reproductive hormones in men. Environ Res 2003;93(1):20-30.

34 Al-Hiyasat AS, Elbetieha AM, Darmani H Reproductive toxic effects of ingestion of sodium fluoride in female rats. Fluoride 2000;33(2):79-84.

35 Diouf M, Cisse D, Lo CM, Ly M, Faye D, Ndiaye O. Pregnant women living in areas of endemic fluorosis in Senegal and low birth weight newborns: case-control study. Rev Epidemiol Sante Publique 2012;60(2):103-8. [in French].

36 Susheela AK, Mondal NK, Gupta R, Ganesh K, Brahmankar S, Basin S, Saxena A. A novel and effective interventional approach for prevention and control of anemia in pregnancy and low birth weight babies. Presentation at the Global Maternal Health Conference organized by Bill and Melinda Gates Foundation; Engender, USA; and Public Health Foundation of India. Aug 30-Sept 1, New Delhi, India.