

## In this issue

### Light reactions of photosynthesis

Photosynthesis begins with light absorption within femtoseconds in pigment-protein complexes (antenna), followed by excitation energy transfer to reaction centres, where the light energy is converted into chemical energy within picoseconds. This then leads to electron transfer within milliseconds. The end products (the reducing power and ATP) of these reactions are then used to produce food and molecular oxygen in plants, algae and cyanobacteria by the well-known Calvin-Benson cycle. In this issue, Gernot Renger (page 1305) provides an overview of the beautiful machinery where the very first steps of this wonderful process take place. This review deals, in basic physical-chemical terms, with the highly efficient conversion of Sun's energy into chemical energy at the reaction centres, whose structures are now known at atomic level. It includes a discussion of primary reactions not only of oxygenic photosynthetic organisms (cyanobacteria, algae and plants), but of non-oxygenic photosynthetic bacteria. An understanding of how these biological nanomachines operate may lead to applications, which will help us solve the energy crisis facing our world. This review article is a part of an on-going series on 'Photosynthesis and Global Issues'.

### Sea turtles

Studies on threat to sea turtles on account of fishing practices like trawling and gill netting are carried out extensively in the past. However, only limited studies are attempted on the impact of longline fishery on sea turtles in the Indian waters. In view of the rapidly developing longline

fishery in and around Indian waters, for exploitation of the high valued fishes like tunas, swordfishes and allied resources, a study was undertaken (page 1378) to understand the spatial and temporal extent of sea turtle bycatch in the longline fishery operating in the Indian exclusive economic zone (EEZ). The experimental longline fishing conducted by the tuna longline research vessels along the Eastern and Western EEZ during 2005-2008 recorded an average bycatch hooking rate of 0.108% sea turtles in every thousand hooks operated. This level of bycatch hooking rate is perhaps lower than the bycatch rate reported from different



parts of the World Oceans. However, the study advocates the importance of more research and continuous monitoring of the incidental bycatch of the different turtle species and addresses the issue of possible stock depletion due to longlining, a relatively new fishing technology to the region. The importance of developing mitigation devices, modification to the fishing gear and creating awareness among the fishermen for avoiding areas of high sea turtle concentration and their migration route or safe release of sea turtles caught, etc. are some of the conservation and management policies that could be adopted to reduce the fishing mortality of the turtles. The need

for cooperative research among the coastal nations to conserve the migratory sea turtle species is highlighted.

### Anaemia in pregnancy: easily rectifiable problem

Anaemia in pregnancy is a serious public health problem. It leads to maternal mortality unless the women with low haemoglobin (<5.0 g/dl) are given blood transfusion. The Infant Mortality Rate (IMR) is in the range of 63-75 per 1000 live births.

A. K. Susheela *et al.* (page 1320) report an effective interventional approach to control anaemia in pregnant women; the procedure is simple and easily implementable in health delivery outlets. The focus is on withdrawing fluoride from ingestion. If urine fluoride is more than 1.0 mg/l, one need to take adequate care that fluoride-containing items are not consumed; fluoridated toothpaste is substituted with Ayurvedic pastes with less fluoride content.

A series of adverse reactions of fluoride consumption are known to occur in cells and tissues: (i) Production of less number of erythrocytes (RBCs)/abnormal erythrocytes by the bone marrow and other haemopoietic tissues due to inadequate thyroid hormonal stimulus; (ii) Reduced blood folic acid activity; (iii) Reduced microbial growth in the gut and inhibits vitamin B<sub>12</sub> production by the probiotics; (iv) Loss of microvilli (brush border) in the intestinal lining, resulting in non-absorption of nutrients for haemoglobin biosynthesis. The merit of the procedure lies in reversing the damages caused, and within a few days upon withdrawal of fluoride.

# Effective interventional approach to control anaemia in pregnant women

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**Anaemia in pregnancy and low birth weight babies, a serious public health problem, troubles India and several other nations. This article reports the results of a approach to address the issue. Women up to 20 week pregnancy with haemoglobin (Hb) 9.0 g/dl or less, those with urinary fluoride beyond 1.0 mg/l and not suffering from any other ailments, were selected. Out of the 205 pregnant women attending antenatal clinics (ANCs) during 1st and 2nd trimesters, the sample and control groups were selected through computerized random sampling procedure. Ninety pregnant women formed the sample group and 115 formed the control group. The sample group was introduced to two interventions, viz.: (1) removal of fluoride from ingestion through drinking water, food and other sources, (2) counselling based intake of essential nutrients, viz. calcium, iron, folic acid, vitamins C, E and other antioxidants through dairy products, vegetables and fruits. No intervention was introduced for the control group. Sample and control groups were monitored for urinary fluoride and Hb until delivery during their visits to ANC. Birth weight of the babies were recorded from the labour room register. Results reveal that (1) the urine fluoride levels decreased in 67% and 53% of the pregnant women respectively, who attended ANCs during 1st and 2nd trimester of pregnancy. (2) An increase in Hb upon withdrawal of fluoride followed by nutritional intervention in 73% and 83% respectively has also been recorded. (3) Body mass index (BMI) also enhanced. (4) The percentage of pre-term deliveries was decreased in sample group compared to control. (5) Birth weight of babies enhanced in 80% and 77% in sample group women who attended ANC in 1st and 2nd trimester respectively as opposed to 49% and 47% respectively in the control group. (6) The number of low birth weight babies was reduced to 20% and 23% respectively in sample as opposed to 51% and 53% in control groups.**

**Keywords:** Anaemia, haemoglobin, low birth weight, pregnancy, urine fluoride.

INDIA and many other nations face a serious problem of anaemia in pregnancy, resulting in low birth weight

babies. The Government took cognizance of the issue in 1970 (ref. 1). Considering that the diet is deficient in iron requirement for haemoglobin (Hb) biosynthesis, the decision to supplement iron along with folic acid to pregnant women visiting antenatal clinics (ANCs) was implemented throughout the country. Iron (60 mg) and folic acid (500 µg) was administered orally as a tablet for 90 days during the 1st and 2nd trimesters. Based on a review conducted during 1985–86 in 11 states in India, by the Indian Council of Medical Research (ICMR), it was observed that the intervention had not made any difference in the haemoglobin level/anaemia in pregnant women of more than 37 weeks of gestation<sup>2</sup>. This led to changing the strength of the tablet to 100 mg iron and 500 µg folic acid and this has been in vogue since 1992 (ref. 3).

The problem continues to plague the country even as late as 2009. According to UNICEF 2008 Report, the highest percentage of low birth weight babies below 5 years of age, namely 43%, is in India. The prevalence in South Asia is 42%; it is 35% in the least developed countries; 26% in developing countries and 25% in the world<sup>4</sup>. The situation is alarming and requires to be addressed with a relook at the risk factors, other than dietary deficiencies, parasitic infestation, urinary tract infection and malaria. The literature is voluminous. Consequences of iron deficiency anaemia in pregnant women could result in (i) increased maternal mortality and morbidity, (ii) increased foetal morbidity and mortality, (iii) increased risk factor of low birth weight babies resulting in brain and thyroid gland damage which may be irreparable<sup>5–8</sup>.

While dealing with iron deficiency anaemia, if rectification is approached through iron and folic acid supplementation besides diet counselling, it is important to address factors enhancing non-haeme iron absorption, viz. vitamin C and low pH achieved through lactic acid production. The inhibitors of non-haeme absorption such as phytates, polyphenols, tannins besides soya protein are to be avoided<sup>9</sup>. While counselling, if the women are expected to practise the interventions, the information should be packaged in a manner that can be put into practice with ease. It has been our observation that any number of IEC (information, education and communication) materials printed and distributed is unlikely to achieve the same results as those that can be achieved by discuss-

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ing and explaining the requirements of dietary changes to the pregnant women with concern and compassion.

Iron cum folic acid supplementation and diet counselling offered by ANCs ought to have corrected iron deficiency anaemia in India; but it has not happened as desired in spite of massive efforts and investments. Breyman<sup>10</sup> is of the view that haemoglobin alone is insufficient to guide management of pregnant women with iron deficiency and anaemia. In Nepal, iron and folic acid supplementation reduced the incidence of low birth weight by 16%. Supplementation of 14 micronutrients including iron, folic acid and zinc reduced low birth weight by 14%, thus confirming no added advantage of multiple micronutrients over iron and folic acid<sup>11</sup>. Other factors need to be investigated, and one such factor is fluoride intake. Fluoride causes serious damage to the gastrointestinal (GI) mucosa by destroying microvilli resulting in non-absorption of nutrients from the diet<sup>12-15</sup>. Fluoride is also known to destroy erythrocytes, thereby contributing to loss of haemoglobin which results in anaemia<sup>16,17</sup>.

Fluoride is a toxic chemical and it is a risk factor for thyroid hormone production in children when the exposure to fluoride occurs during intrauterine growth period<sup>18</sup>. Fagin's report in *Scientific American* 'on second thoughts about fluoride' during 2008 is a warning to all concerned as he has revealed the risk of fluoride causing disorders affecting teeth, bone, brain and thyroid gland<sup>19</sup>. As early as 1979, US dairy scientists have reported that thyroxine and triiodothyronine in serum decreased with increasing urinary fluoride in cattle. Cattle affected with fluorosis developed hypothyroidism and anaemia<sup>17</sup>. Thyroid hormone status in married women prior to conception may therefore be required to be assessed.

Keeping in view the information available on fluoride and fluoride toxicity, a protocol was designed with prime objective of controlling anaemia in pregnant women with high fluoride intake who are on routine iron and folic acid supplementation. ANC approach was preferred, as the study could be executed and monitored until delivery.

## Material and methods

A general hospital located in the National Capital Territory of Delhi (NCTD), India, where women from the lower strata of society attend ANCs, was identified and necessary clearance to work in the hospital was obtained from the administration and the head of the obstetrics and gynaecology (OBGY) department. The project was launched in 2005. A total of 2055 pregnant women were screened over a period of 2 years and 6 months. Pregnant women who were more than 20 weeks into gestation and those suffering from diabetes, tuberculosis, bleeding during pregnancy, high blood pressure, HIV AIDS, malaria and other medical problems were excluded. Only those women who were anaemic (Hb  $\leq$  9.0 g/dl) were consi-

dered for investigations. These exclusion criteria reduced the target population size to 249.

### Computerized random sampling procedure

The study population was grouped into sample and control using computerized random sampling procedure. The sample and control groups were subjected to the following three laboratory tests: (i) Hb test, (ii) Fluoride content in drinking water, and (iii) Fluoride content in urine sample.

Hb content was measured using HemoCue 201+, a digital, portable unit, so that the women can see the results instantaneously. HemoCue is used extensively for field-based studies<sup>20,21</sup>.

The fluoride test was conducted in both drinking water and urine samples based on the method of Hall *et al.*<sup>22</sup>. The equipment used is Ion meter model, ION 85 Ion Analyser, Radiometer, Copenhagen.

Information on the following confounding factors was gathered using a specially designed proforma for the dietary regime, economic status, literacy status of the women, employment status, first pregnancy or the women had earlier issues, any miscarriage/intra-uterine death, history of previous or present ailment; and consumption of iron and folic acid tablets provided by the hospital.

Height and weight measurements were made. Blood pressure measurements from hospital records were transferred to the study proforma. Wherever any of these were missing, measurements were done by the investigating team. The information thus collected was used for analysis.

*Sample population:* All pregnant women in the sample population were anaemic (Hb  $\leq$  9.0 g/dl, though anaemia is denoted when Hb is  $<$ 11.0 g/dl, anaemic women with Hb from 9.0–5.0 g/dl were chosen for the study), with urinary fluoride more than 1.0 mg/l during the first visit to the ANC. Only those with Hb level up to 5.0 g/dl were selected. The sample population was subjected to two intervention procedures.

(i) *Intervention procedure 1: to avoid consumption of fluoride containing water and food items.* Counselling was provided to avoid consumption of fluoride containing food, water and other substances for arrest of injury to cells/tissues and for enabling regeneration of the damaged GI mucosa.

Upon reviewing the data on fluoride content in drinking water and if fluoride was beyond normal limits (more than 1.1 mg/l, the national guideline for fluoride in drinking water is 1.0 mg/l, as the upper limit, less the better), the subjects were shifted to an existing safe source of water in their neighbourhood for collecting water for drinking and cooking purposes. In the event that drinking water fluoride was below 1.0 mg/l and therefore safe but urinary fluoride was high (reference range 0.1–1.0 mg/l), the source(s) of fluoride was traced through retrieving

information on diet and dietary habits to find the food items consumed and the sources known to contain high fluoride.

Consumption of all items including food enriched with fluoride was withdrawn. Items leading to high fluoride intake are (i) use of black rock salt ( $\text{CaF}_2$ ) with 157 ppm fluoride (analytical data 2007 – unpublished) in cooking – black rock salt is toxic and harmful to health; (ii) all items including Indian street food (junk food) enriched with black rock salt to enhance the aroma and tangy taste; (iii) black tea without milk, *churans*, and toothpaste with high fluoride and (4) salted snacks and spices smeared with rock salt.

Counselling to avoid drinking water and food containing fluoride is an intervention that the pregnant women in sample group were introduced to for rectifying the damage caused to the GI mucosa<sup>23</sup>. The mucosa is known to regenerate within a short interval of 10–15 days upon withdrawal of fluoride and absorption of nutrients including orally administered iron and folic acid tablets would commence thereafter.

(ii) *Intervention procedure 2: promotion of intake of essential nutrients through diet.* Diet counselling was given for promoting adequate intake of essential nutrients, viz. calcium, iron, folic acid, vitamins C, E and other antioxidants for repair and maintenance of the damaged cells and tissues as well as for enhancing rise in Hb.

The focus of counselling was on the importance of consuming a nutritive diet and the need to acquire essential nutrients through dairy products, vegetables and fruits. Antioxidant intake is essential since fluorine, being a powerful oxidizing agent, produces oxygen free radicals and these need to be eliminated from the system. Vegetables and fruits rich in antioxidants act as scavengers for eliminating free radicals and restoring the system to normalcy. In addition to counselling, a little pictorial booklet in local language on dietary details was also given to pregnant women.

The pregnant women followed the advice as it involved simple recipes, affordable by the poor, rural and urban population. Besides, they found the information easy to practice. It was also indicated that the information imparted was not only for the benefit of one member, and that other members of the family, could consume the same food. The counselling was for improving the health of all members of the household and not ‘pregnancy specific’. Such advice is found more acceptable to the women as food is cooked for the entire family and not particularly for one individual.

Distribution of IEC materials followed by discussion with concern and compassion had an impact on the sample group.

### *Control population*

The control population is the same in all respects as the sample population with Hb  $\leq 9.0$  g/dl and urinary fluoride

$> 1.0$  mg/l. The only difference is that they were not counselled for intervention procedures 1 and 2.

*Monitoring the subjects:* The sample and control population were monitored until delivery by testing for urinary fluoride and haemoglobin during every visit to the ANC. The pregnant women were informed about their Hb levels. A target number of Hb to be attained, prior to the next visit to the ANC was also indicated. It turned out that a few women came only once or twice due to personal reasons while the majority visited the ANC 3–7 times prior to delivery. Those who came only once or twice, were not included for reporting the data.

Some women did not come to the same hospital for delivery; but went to different hospitals closer to their homes. The investigating team was alert to this fact, since every woman was followed up through telephone calls and information about the mother and baby was being monitored. The information collected and recorded in the labour room register was checked and all relevant information transferred to the project proforma.

## **Results and discussion**

Various sources of fluoride starting from drinking water (naturally contaminated with fluoride), food, food products and beverages like black tea (without milk) were identified and withdrawn. The impact of such an approach along with promotion of nutritive diet, and the outcome in 90 sample group women who attended ANC during 1st and 2nd trimester of pregnancy are reported in Tables 1 and 2. As there are reports to suggest that iron deficiency during 1st trimester results in significant reduction in foetal growth<sup>24,25</sup> and not beyond, the focus was to evaluate the results trimester-wise.

Withdrawal of fluoride source(s) possibly resulted in the expected regeneration of GI mucosa and microvilli, and this in turn enhanced the absorption of nutrients as evidenced by the reduction in urinary fluoride followed by rise in Hb levels.

Those women who either withdrew or reduced fluoride intake irrespective of whether they attended the ANC during the 1st or 2nd trimester of pregnancy not only showed reduced levels of urinary fluoride but also benefited in terms of raising their Hb levels. This is amply evident from the data reported in Tables 1 and 2. In sample group, the urinary fluoride was reduced from  $2.082 \pm 1.058$  to  $1.628 \pm 1.631$  in women who attended ANC during 1st trimester of pregnancy, showing a concomitant rise in Hb from  $8.2 \pm 0.9$  to  $10.8 \pm 2.0$ . In a similar manner, when urinary fluoride is reduced from  $1.939 \pm 1.122$  to  $1.441 \pm 0.894$ , the Hb enhanced from  $8.4 \pm 0.8$  to  $10.1 \pm 1.8$  in the women who attended ANC first time during 2nd trimester. Reduction in urinary fluoride is recorded in 20 out of the 30 women of the sample

**Table 1.** Urine fluoride, haemoglobin of pregnant women of sample and control groups during the initial and prior to delivery in those who first attended ANC during the 1st trimester of pregnancy (10–15 week) and birth weight of babies

	Urinary fluoride level (UFL) (mg/l)			Haemoglobin (Hb) (g/dl)			Birth weight of babies (kg)	
		UFL initial	UFL prior to delivery		Hb initial	Hb prior to delivery	Low birth wt (<2.5 kg)	Normal birth wt (≥2.5 kg)
Sample <i>n</i> = 30	Mean ± SD	2.082 ± 1.058	*1.628 ± 1.631	Mean ± SD	8.2 ± 0.9	**10.8 ± 2.0	Mean ± SD 2.21 ± 0.17	***2.94 ± 0.27
	Range	1.082–6.256	0.254–7.749	Range	5.7–9.0	5.4–13.3	Range 1.95–2.45	2.5–3.5
		Reduction in UFL in 20/30 = 67%			Rise in Hb in 22/30 = 73%		Normal birth weight born in 24/30 = 80%	
							Low birth weight born in 6/30 = 20%	
Control <i>n</i> = 37	Mean ± SD	1.617 ± 1.158	*1.702 ± 1.709	Mean ± SD	8.3 ± 0.9	**9.3 ± 1.5	Mean ± SD 2.00 ± 0.58	***2.76 ± 0.27
	Range	0.403–4.094	0.263–8.768	Range	5.6–9.0	6.3–12.0	Range 1.3–2.43	2.5–3.3
		Reduction in UFL in 18/37 = 49%			Rise in Hb in 22/37 = 59%		Normal baby weight 18/37 = 49%	
							Low birth weight 19/37 = 51%	

\**P* > 0.01 (nonsignificant); \*\**P* < 0.001; \*\*\**P* < 0.1; SD, Standard deviation.

**Table 2.** Urine fluoride, haemoglobin of pregnant women of sample and control groups during the initial and prior to delivery in those who first attended ANC during the 2nd trimester (16–20 week) and birth weight of babies

	Urinary fluoride level (UFL) (mg/l)			Haemoglobin (Hb) (g/dl)			Birth weight of babies (kg)	
		UFL initial	UFL prior to delivery		Hb initial	Hb prior to delivery	Low birth wt (<2.5 kg)	Normal birth wt (≥2.5 kg)
Sample <i>n</i> = 60	Mean ± SD	1.939 ± 1.122	*1.441 ± 0.894	Mean ± SD	8.4 ± 0.8	**10.1 ± 1.8	Mean ± SD 2.24 ± 0.19	***2.94 ± 0.32
	Range	0.130–5.464	0.549–5.446	Range	6.3–9.0	5.6–13.2	Range 1.87–2.48	2.5–3.89
		Reduction in UFL in 32/60 = 53%			Rise in Hb in 50/60 = 83%		Normal birth weight 46/60 = 77%	
							Low birth weight 14/60 = 23%	
Control <i>n</i> = 78	Mean ± SD	1.364 ± 1.038	*1.723 ± 0.986	Mean ± SD	8.0 ± 1.2	**9.1 ± 1.6	Mean ± SD 2.01 ± 0.64	***2.72 ± 0.21
	Range	0.231–6.134	0.387–4.778	Range	5.1–9.0	5.1–12.2	Range 1.25–2.48	2.5–3.3
		Reduction in UFL in 29/78 = 37%			Rise in Hb in 42/78 = 54%		Normal birth weight in 37/78 = 47%	
							Low birth weight in 41/78 = 53%	

\**P* > 0.01; \*\**P* < 0.001; \*\*\**P* < 0.0001; SD, Standard deviation.

group of trimester 1, and 32 out of 60 women of trimester 2, which is about 67% and 53% respectively. The rise in Hb is recorded in 22 out of the 30 women which is about 73% and 50 out of the 60 women which is about 83%. This is a significant change arising as a result of withdrawal or reduction in fluoride ingestion followed by a dietary improvement by the pregnant women.

The women in the control group (1st trimester of pregnancy) on the contrary reveal a rise in urinary fluoride instead of reduction from 1.617 ± 1.158 to 1.702 ± 1.709. The Hb has been minimally increased, i.e. 8.3 ± 0.9 to 9.3 ± 1.5. In a similar manner, for women in 2nd trimester of pregnancy, the urinary fluoride was enhanced from 1.364 ± 1.038 to 1.723 ± 0.986; the Hb level had also minimally increased from 8.0 ± 1.2 to 9.1 ± 1.6.

In the control, urinary fluoride reduction is recorded in 49%, i.e. 18 out of the 37 women of 1st trimester. This shows 51% women showed rise or no change in urinary fluoride. Similarly in women of 2nd trimester, 37% showed reduction in urinary fluoride level (UFL), i.e. reduction is recorded in 29 out of the 78 women investigated during 2nd trimester. This again showed 63% women revealed rise/no change in urinary fluoride.

In the control group, rise in Hb is observed in 22 out of the 37 women of the 1st trimester, i.e. 59%; and Hb rise is in 42 out of the 78 women of 2nd trimester which is only 54% of women who participated in the study.

The next obvious issue probed into is whether the rise in Hb in pregnant women has in any manner affected the birth weight of the babies. In the 30 pregnant women of trimester 1, the initial Hb range being 5.7–9.0 g/dl, the practice of interventions led to rise in Hb in the range 5.4–13.3 g/dl prior to delivery. The rise in Hb is recorded in 22 out of the 30 women investigated, which works out to about 73% of the study group. How many among the 30, gave birth to normal birth weight babies and how many delivered low birth weight babies?

Table 1 shows that 24 out of the 30 women delivered normal birth weight babies (i.e. 80%), whose birth weight ranged from 2.5 to 3.5 kg (mean 2.94 ± 0.27). Among the sample group, 6 women out of the 30 (i.e. 20%) delivered low birth weight babies; birth weight range was from 1.95 to 2.45 kg (mean 2.21 ± 0.17).

Reviewing the outcome of the interventions in sample group (*n* = 60) who attended ANC during 2nd trimester, the Hb range was initially 6.3–9.0 g/dl (mean 8.4 ± 0.8);

after practice of interventions, it was in the range of 5.6–13.2 g/dl (mean 10.1 ± 1.8). The rise in Hb is recorded in 50 out of the 60 women investigated which is to the tune of 83%. The rise in Hb in 83% pregnant women and its impact on the birth weight of the babies is reported in Table 2. It is evident that 46 out of the 60 women delivered normal birth weight babies, i.e. 77%. The normal birth weight of babies ranged from 2.5 to 3.89 kg (mean 2.94 ± 0.32). Among the 60 women, 14 delivered low birth weight babies (i.e. 23%). The weight of the babies ranged from 1.87 to 2.48 kg (mean 2.24 ± 0.19).

The impact of interventions among the pregnant women who attended the ANC first either during 1st or 2nd trimester has been manifested in terms of rise in Hb, resulting in birth of normal birth weight babies to the extent of 80% and 77% respectively. The number of low birth weight babies (<2.5 kg) has been reduced to 20% and 23% respectively. This is considered highly significant (*P* value <0.01 in 1st trimester; *P* value <0.0001 in 2nd trimester, Tables 1 and 2).

In the women of the control group of 1st trimester, the initial Hb range recorded is 5.6–9.0 g/dl (mean 8.3 ± 0.9) and prior to delivery the changes recorded in Hb is 6.3–12.0 g/dl (mean 9.3 ± 1.5). The rise in Hb was observed in 22 out of the 37 which is about 59%. Similarly, among the control group women who attended the ANC during the 2nd trimester, the Hb range during the initial phase was 5.1–9.0 g/dl (mean 8.0 ± 1.2) and prior to delivery, the range was 5.1–12.2 (mean 9.1 ± 1.6). Among the 37 women of the 1st trimester, 18 delivered normal birth weight babies (i.e. 49%), whereas among the 78 control group women of 2nd trimester, 37 delivered normal birth weight babies (i.e. 47%). In the control group of trimesters 1 and 2, 51% and 53% babies born respectively are of low birth weight; birth weights ranging from 1.3 to 2.43 kg (mean 2.00 ± 0.58) and 1.25 to 2.48 (mean 2.01 ± 0.64) (Tables 1 and 2).

Tables 1 and 2 provide evidence that fluoride ingestion arrests the absorption of nutrients including orally administered iron and folic acid. Therefore, withdrawal of fluoride from ingestion does provide beneficial results in

controlling anaemia and improving the birth weight of babies.

The interventions have also been of advantage to the women in terms of increasing body mass index (BMI)<sup>26</sup> and reducing pre-term deliveries<sup>25</sup>. These results are reported in Tables 3 and 4.

The data reveals that among the 90 women in the sample group, 22 had BMI at the initial stages < 18.5 (range 14.4–18.4), suggesting prevalence of underweight/under-nutrition. However, prior to delivery the number in this category was reduced to 2. This is an impact of interventions. Initially there were 63 women, with BMI 18.5–24.9 (i.e. normal range) but prior to delivery improvement was observed in 67 women, yet again showing impact of interventions. In the BMI category, 25.0–29.9 is normally considered overweight but in pregnancy the rise in weight is desirable. Initially there were 5 women in the category; this number was increased to 21 prior to delivery. In the category of BMI ≥ 30.0 which is obese, there were none, which is also a healthy sign.

The impact of interventions on gestation period at delivery is an added advantage to the sample group women. Table 4 shows that pre-term delivery, i.e. <34 weeks of gestation, was recorded in 2 out of 90 (i.e. 2%); whereas it is 9 out of 115 in the control (i.e. 8%); four times more in the control group who were not introduced to interventions. Delivery between 34 and 37 weeks of gestation was 30% and 42% respectively, in sample and control groups. Delivery at gestation period >37 weeks was 68% in sample and 50% in control groups. There were two stillbirths in control and none in sample group. The overall benefits accrued through the intervention procedures have undoubtedly been extraordinary.

### Statistical analysis

The overall interventional impact was assessed through different statistical parameters. Very significant reduction in urinary fluoride level (UFL) along with substantial gains in the Hb level and BMI are observed in the sample group subjected to interventions. The quantified gains are

**Table 3.** Impact of intervention(s) on body mass index (BMI)

BMI categories	Body mass index							
	Initial				Prior to delivery			
	<18.5	18.5–24.9	25.0–29.9	≥ 30.0	<18.5	18.5–24.9	25.0–29.9	≥ 30.0
Sample group <i>n</i> = 90	<i>n</i> = 22	<i>n</i> = 63	<i>n</i> = 5	<i>n</i> = nil	<i>n</i> = 2	<i>n</i> = 67	<i>n</i> = 21	<i>n</i> = nil
BMI range	14.4–18.4	18.5–24.7	25.2–26.0	–	18.2–18.4	18.8–24.8	25.3–28.9	–
Mean ± SD	17.2 ± 1.3	20.8 ± 1.8	25.7 ± 0.4	–	18.3 ± 0.1	22.3 ± 1.6	27.1 ± 1.2	–
Control group <i>n</i> = 115	<i>n</i> = 36	<i>n</i> = 73	<i>n</i> = 6	<i>n</i> = nil	<i>n</i> = 4	<i>n</i> = 88	<i>n</i> = 20	<i>n</i> = 3
BMI range	15.6–18.3	18.5–24.5	25.2–29.0	–	17.3–18.2	18.5–24.9	25.0–28.2	30.1
Mean ± SD	17.2 ± 0.9	20.9 ± 1.6	27.3 ± 1.7	–	17.8 ± 0.4	22.1 ± 1.7	26.2 ± 0.9	30.1 ± 0.0

SD = Standard deviation.

**Table 4.** Gestation period at delivery

	Gestation period		
	< 34 Weeks (pre-term delivery)	34–37 Weeks (term delivery)	> 37 Weeks (full-term delivery)
Sample $n = 90$	$n = 2$	$n = 27$	$n = 61$
Mean $\pm$ SD	33.0 $\pm$ 0.0	Mean $\pm$ SD 36 $\pm$ 1.1	Mean $\pm$ SD 39.3 $\pm$ 1.1
Range (weeks-days)	33.0–33.0	Range (weeks-days) 34.0–37.0	Range (weeks-days) 38.0–42.0
	< 34 weeks (pre-term delivery) in 2/90 = 2%	34–37 weeks (term delivery) in 27/90 = 30%	> 37 weeks (full-term delivery) in 61/90 = 68%
Control $n = 115$	$n = 9^*$	$n = 48^{**}$	$n = 58^{***}$
Mean $\pm$ SD	31.2 $\pm$ 4.6	Mean $\pm$ SD 36 $\pm$ 1.1	Mean $\pm$ SD 39.2 $\pm$ 1.0
Range (weeks-days)	31.0–33.0	Range (weeks-days) 34.0–37.0	Range (weeks-days) 38.0–41.0
	< 34 weeks (pre-term delivery) in 9/115 = 8%	34–37 weeks (term delivery) in 48/115 = 42%	> 37 weeks (full-term delivery) in 58/115 = 50%

\*One spontaneous abortion at 19 weeks. \*\*\*Two pre-term stillbirth at 30th and 31st weeks. SD, Standard deviation.

far greater in this group as compared to the control group that was not subjected to interventions. Additionally, the sample groups had higher average baby weight as compared to the control group. The proportion of babies born with normal weight was also higher in the sample group.

Quantifications of all these parameters suggest that interventions for control of anaemia in pregnant women have been highly impactful. The benefit accrued to the families is highly significant and this was possible due to elimination of fluoride and promotion of a nutritive diet rich in essential nutrients.

The results have been as critically evaluated as possible, with a view to identify the major detrimental and confounding factors statistically. The goal is to understand the merits of the results better so that such a process can be replicated/scaled-up within the country and elsewhere in the world.

We conjectured the following two hypotheses and validated them statistically: (1) Pregnant women with high urinary fluoride have low Hb levels. (2) Reduction in urinary fluoride of pregnant women results in increase in Hb.

The analysis indicated a positive impact of the withdrawal of urinary fluoride on Hb levels in pregnant women. The analysis also reveals that our hypothesis (2) is statistically valid. It signifies that interventions have played a key role in increasing Hb levels with lowering of urinary fluoride in the pregnant women, an approach hitherto unknown.

## Evaluation of data based on hypotheses 1 and 2

At the overall level, it is observed that urinary fluorides were reduced to normal levels in 35% sample group cases as against 26% in the control group (Table 5). It should be pointed out that in statistical evaluation, the data analysis is based on the total number in sample and control groups; there is no bifurcation into trimesters 1 and 2.

This observation alone is misleading if conclusions are made regarding an impact of the intervention programme on account of the following facts.

- UFLs were observed to have fluctuated drastically in the intervening periods due to non-adherence to dietary guidelines.
- In table, we observe only the difference between UFLs during the first and the last patient visits.
- Changes in Hb and BMI levels are equally critical for any conclusions to be made about the interventions.
- It will also be important to find the factors detrimental to the reduction of UFL and Hb through interventions.

It is, therefore, important to assess the impact of changes in UFLs in conjunction with changes in Hb and BMI levels. We have made an attempt to set up a few relevant hypotheses and justify them using the observed data.

### Hypothesis 1

Pregnant women with high urinary fluoride have low Hb levels. It is observed (Table 6) that Hb levels improved to normalcy in 71% of the sample cases as against 37% in the control group. Clearly some strong impact of interventions to control urinary fluoride and thereby Hb levels is visible. Further, studying UFLs in those cases where Hb levels were low would provide a better understanding of the impact of interventions.

Percentage of pregnant women with high urinary fluoride and low Hb levels has decreased from 99% at their first visit to 85% prior to delivery in the sample group (Table 7). It means a substantial 14% of these women have improved their Hb levels. On the other hand, comparable women in the control group indicated an equivalent rise in their percentage (15%; from 54% at first visit to 69% prior to delivery). This clearly indicates a very

**Table 5.** Percentage distribution of pregnant women by urinary fluoride levels (UFLs)\*

	Sample group (90)			Control group (115)		
	High UFL (%)	Normal UFL (%)	Total (%)	High UFL (%)	Normal UFL (%)	Total (%)
On first visit	99	1	100	54	46	100
Prior to delivery	64	36	100	28	72	100
Percentage change	-35	+35		-26	+26	

UFL (normal) means urinary fluoride less than 1.0 mg/l on the 1st visit. In Materials and methods, all pregnant women have UFL more than 1.0 mg/l.

**Table 6.** Pregnant women by Hb levels (% distribution)

	Sample group (90)			Control group (115)		
	Low Hb (%)	Normal Hb (%)	Total (%)	Low Hb (%)	Normal Hb (%)	Total (%)
On first visit	100	0	100	100	0	100
Prior to delivery	29	71	100	63	37	100
Percentage change	-71	+71		-37	+37	

**Table 7.** Pregnant women by UFLs with low Hb (% distribution)

	Sample group (26/90)			Control group (72/115)		
	High UFL (%)	Normal UFL (%)	Total (%)	High UFL (%)	Normal UFL (%)	Total (%)
On first visit	99	1	100	54	46	100
Prior to delivery	85	15	100	69	31	100
Percentage change	-14	+14		+15	-15	

positive correlation between withdrawal of urinary fluorides and the rise in Hb levels of pregnant women.

*Hypothesis 2*

Interventions do not result in significant increases in Hb levels with reduction in UFLs of pregnant women. Interventions, either direct (withdrawal of drinking water source and food contaminated with fluorides) or indirect (advice on proper dietary methods) are intended to be preventive in nature. A simple correlation analysis (Table 8) indicates that interventions have acted as huge stimuli for improved Hb levels in sample group of pregnant women as compared to the control group. Correlation between UFLs and Hb levels increases (in absolute terms) from -0.14 to -0.39 for the sample group between first and last visits of the pregnant women. Starting from a negative relationship (corr = -0.14) between the two factors at the first visits, it strengthens by a margin of 25 points in the negative direction. A similar differential for the control group is only 17 points. This differential is highly significant and nullifies the hypothesis of ‘no impact of intervention’. It signifies that interventions have played a key role in increasing Hb levels with lowering of UFLs (Table 9; Figures 1 and 2) in the pregnant women.

**Confounding factors detrimental to improvement in Hb levels**

It is observed that despite subjecting the pregnant women to interventions, in 29% (26 cases) of the sample group, Hb levels remained below normal (Table 6). Therefore, it is pertinent to study the factors that caused this adversity. Amongst the prominent factors that we could consider and for which data was collected were – family income, subject’s age, subject’s literacy, subject’s occupation, husband’s occupation, subject’s locality type, subject’s family size, etc. For our analysis, as required and appropriate, data had to be either regrouped (e.g. create age groups instead of age in years) or transformed (e.g. compute per capita income instead of absolute family income).

*Data transformation*

Understanding data transformations and any assumptions is equally important to appreciate the analytical results. While the important ones are listed here, others are non-critical.

*HB\_LowLow:* A dummy variable for low Hb level at the first contact remaining low at the last contact. Vari-



**Table 8.** Correlation analysis

	Sample group	Control group
Correlation between UFL and Hb levels – first visit	-0.14	0.00
Correlation between UFL and Hb levels – prior to delivery	-0.39	-0.17
Average number of visits	4.13	3.55
Number of valid observations	90	115

**Table 9.** Summary of interventional impact

	With intervention			Without intervention		
	At 1st visit	Prior to delivery	Percentage change (%)	At 1st visit	Prior to delivery	Percentage change (%)
Median* Hb level	8.65	10.60	22.54	8.60	9.40	9.30
Median UFL	1.72	1.13	-34.22	1.14	1.34	17.65
Median BMI level	19.80	23.28	17.58	20.15	22.65	12.41
Average intervention period (days)		120.0			111.0	
Average baby weight at birth (kg)		2.78			2.44	
Percentage babies born with $\geq 2.5$ kg		78%			48%	
Percentage patients with decline in UFLs		73%			40%	
Number of valid observations		90			115	

\*Median is a point above/below which 50% of the cases lie.

able HB\_LowLow is equal to 1 if low Hb remains low; and is equal to 0 otherwise.

**Occupation:** Subject's occupation is grouped as housewife = 1, unskilled worker = 2, other workers (all cases unclassified as 1 or 2) = 3. This grouping incorporates the level of occupation, 1 being low and 3 being high. Wherever subject's occupation was not mentioned, it was assumed to be the same as that of husband's occupation. It is expected that occupation (thus defined) would have a negative relationship with HB\_LowLow.

**Age:** Subject's age is grouped as 1 if age is less than 20 years, 2 if age ranges between 20 and 24 years, 3 if age ranges between 25 and 29 years and 4 if age is 30 years or more. We expect Hb to remain low in lower age groups of subjects.

**Locality:** Subject's locality is grouped as 1 = government colony, 2 = village/developing colony and 3 = slum. It would be expected that subjects from slum areas would be more prone to non-improvements in their Hb levels.

**Literacy:** Subject's and husband's literacy is grouped as illiterate = 1, literate = 2, matric = 3, graduate and above = 4. It is expected that low Hb levels could remain low in literacy groups 1 and 2.

## Identifying factors

Data analysis is aimed at finding the major factors that are detrimental to rise in subject's Hb level. We use logistic regression approach for this purpose. It may be noted that at this stage we are not really keen to know the exact magnitudes of impact of various factors. Therefore,

any inferences from analytical results need to be made in light of the objective.

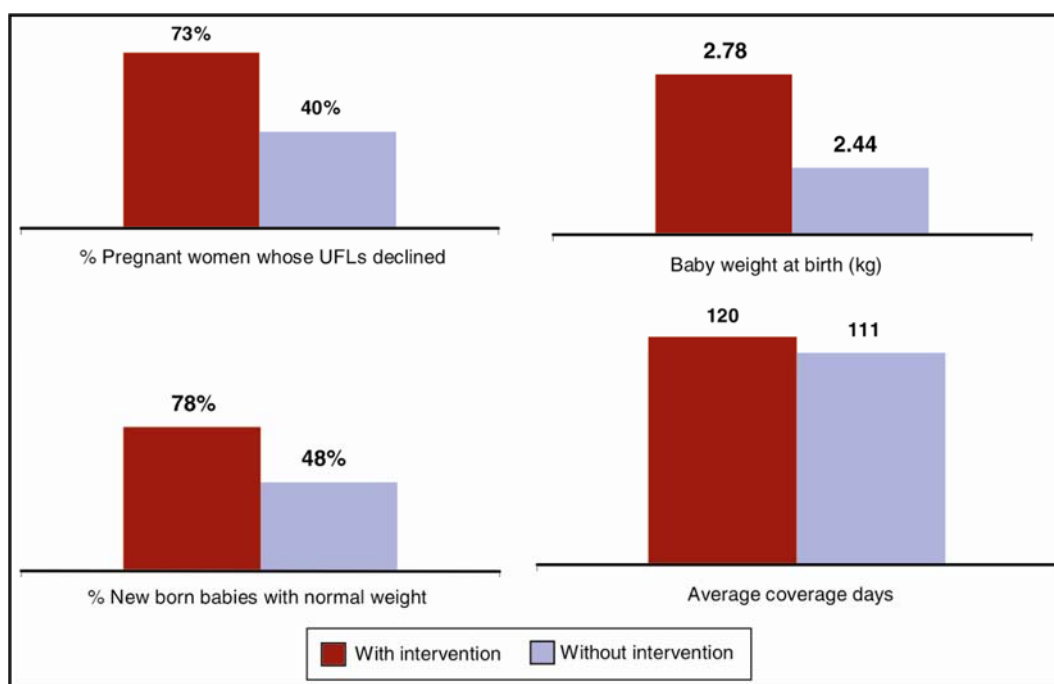
Table 10 presents a snapshot of logistic regression analyses carried out to identify such key factors that are detrimental to growth in subject's Hb level. Prominently, per capita income (which is defined as total family income divided by family size – that takes into account both absolute incomes as well as the family size) and number of times the subject visits the ANC in the hospital for medical and interventional advice are the most significant factors that determine the improvements in Hb levels. On the other hand, most of the demographic characteristics – subject's age, education level (either of the subject or that of the husband), their occupations and the type of locality they lived in – are observed to be non-significant factors. Income in absolute terms is also a non-significant factor towards lower Hb levels. Very interestingly, all the factors show correct direction of impact (signs of coefficients, see Table 10, Logit 1).

It is logical to ask if application of intervention itself is a factor for improvements in Hb levels. This is tested by introduction of a dummy variable for application of intervention (Int\_applied = 1 if subject belongs to the sample group, Int\_applied = 0 if subject belongs to the control group). It is observed that application of intervention is a critical factor for improvements in Hb levels. This also strongly supports our results in earlier sections.

Evaluating the background information retrieved from the pregnant women belonging to the sample and control groups is informative in the sense that in the sample group, members of a household are from a minimum of 2 to a maximum of 12. In the control group the maximum

**Table 10.** Summary of logistic regression results – dependent variable  $Y = HB\_LowLow = 1$

Factor	Logit 1		Logit 2	
	Direction of impact	Significance level	Direction of impact	Significance level
Per capita income (PCI)	Negative	5%	Negative	7%
Subject's age (age_gr)	Negative	Nonsignificant	Negative	Nonsignificant
Subject's occupation (Occ_gr)	Negative	Nonsignificant	Negative	Nonsignificant
Number of contacts (No_visits)	Negative	1%	Negative	1%
Subject's locality type (loc_type_gr)	Negative	Nonsignificant	Negative	Nonsignificant
Intervention applied or not (Int_applied = 1 if yes, = 0 if no)	–	–	Negative	1%



**Figure 1.** Impact of intervention.

number was 7 and not beyond. The income per day varies from a minimum of Rs 83 (US\$ 2.0) to a maximum of Rs 500/day (US\$ 12.5) in the sample group. In control group, the income was a minimum of Rs 66 (US\$ 11.5) to a maximum of Rs 300/day (US\$ 7.5). The per capita income in the sample and control groups is a meagre amount. It ought to be noted that the pregnant women never came alone but were accompanied by family members. To and fro transport charges for two at frequent intervals may be beyond the means of the family. It would appear therefore that the cash availability was a deterrent for frequent hospital visits. This would also suggest that there may be constraints in the purchasing power for food as well and is likely to have an adverse impact.

This analysis also brings out the fact that application of intervention and number of visits by the subject for regular antenatal follow-up are interdependent. This means that, even if a particular subject is put under an interven-

tion programme, the subject may or may not visit for regular antenatal follow-up. However, for success/effectiveness of interventions, subject's number of visits (contacts) for antenatal follow-up should be highly associated with application of intervention. And, we should be expecting this to be so. This clearly indicates that there is a missing link here.

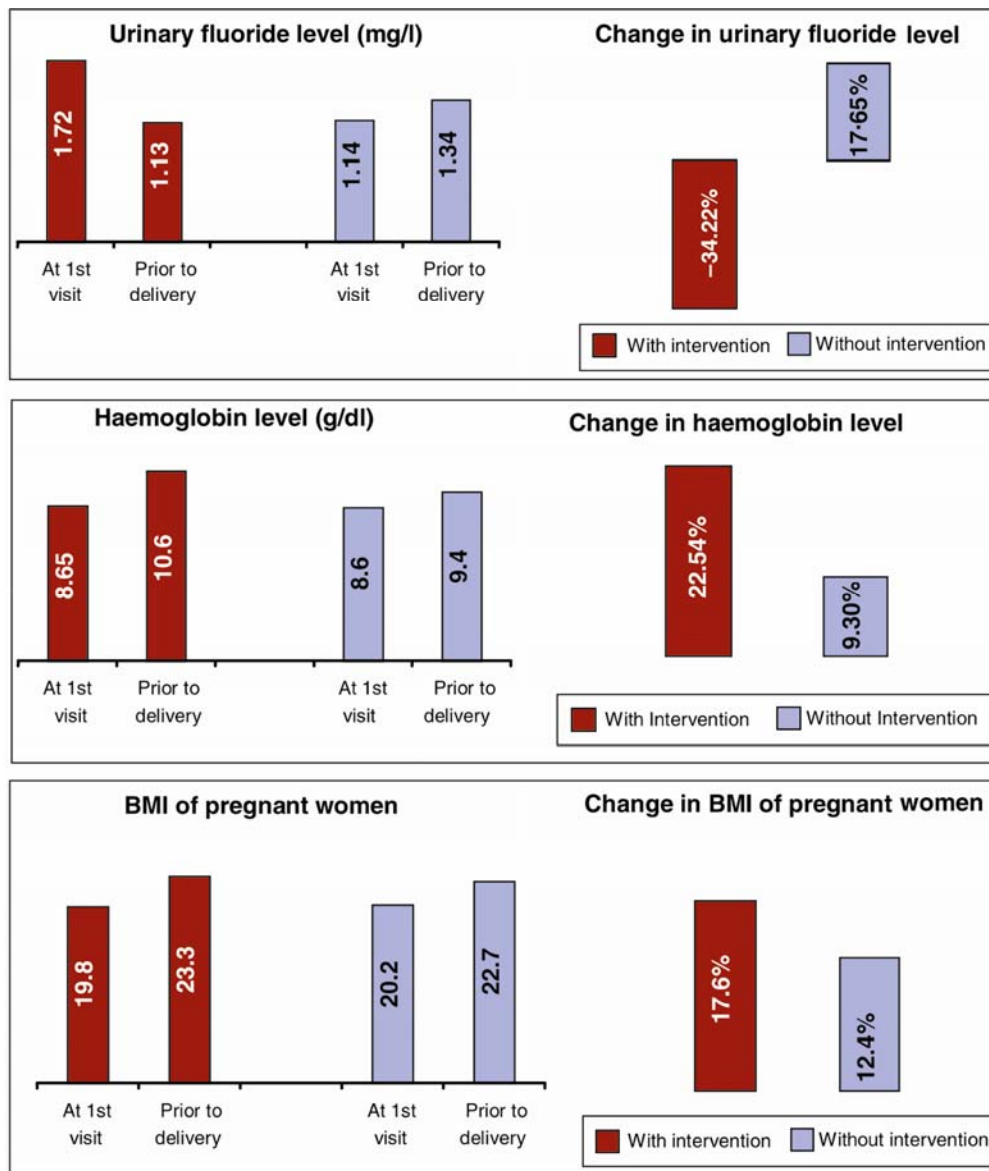
This brings up a question – ‘Are there any specific factors that govern how many times the subject is likely to be present for regular antenatal follow-ups?’ This is tested through a regression analysis. Table 11 depicts a snapshot of factors influencing number of contacts/visits.

It is observed that there is a significant influence of application of intervention on the subject and the locality type where the subject comes from on the number of subject's visits.

Subject's locality type is observed to be a highly significant factor determining number of contacts by the subject. The negative sign indicates that the number of

**Table 11.** Summary of regression analysis (dependent variable  $Y = \text{No\_visits}$ )

Factor	Direction of impact	Significance level
Intervention applied or not (Int_applied = 1 if yes, = 0 if no)	Positive	5%
Subject's age (age_gr)	Positive	Nonsignificant
Subject's occupation (Occ_gr)	Positive	Nonsignificant
Per capita income (PCI)	Positive	Nonsignificant
Subject's locality type (loc_type_gr)	Negative	5%



**Figure 2.** Impact of intervention on UF, Hb and BMI.

contacts declines from government locality to a slum area. We further performed a similar analysis only for subjects from the slum areas and, apparently, none of the factors explains any specific influence on the slum dweller's behaviour. There appears to be more for further investigation.

The first factor (application of intervention) throws up a bit of a confusing result to which we do not have a con-

crete answer at the moment. We could dig deeper into this relationship in a further analysis of our subsequent intervention programmes.

Though fluoridation of a variety of products is still in vogue in many nations, it is difficult to overlook the high percentage of pregnant women exposed to fluoride ingestion and being anaemic in developing countries<sup>24</sup>. A

simple procedure of assessing fluoride in urine and Hb levels in women is adequate to introduce interventions for controlling anaemia in such high percentage of pregnant women. It is evident from the data reported in this communication that maternal and child under-nutrition and anaemia is not necessarily due to insufficient food intake but because of the derangement of nutrient absorption due to damage caused to GI, mucosa by ingestion of undesirable chemical substance, viz. fluoride through food, water and other sources. These aspects have so far been unexplored, and this is the first time such a possibility is investigated and results reported. The findings of this approach in the context of anaemia in pregnancy provide a new path for reducing the burden of disabled and mentally challenged children<sup>25,27</sup> by reducing percentage of low birth weight babies.

In a small percentage in sample group, though the urine fluoride was reduced, Hb did not rise. Low per capita income and not consuming adequate nutritive diet and possibly other reasons such as low thyroid hormone for non-production of adequate RBCs need to be explored.

In conclusion, a novel and effective intervention approach therefore has scope for reducing anaemia in pregnancy and improve birth weight of babies. Fluoride toxicity, as a risk factor was never considered even in the highly endemic regions for fluoride and fluorosis in India and around the globe. This is the first report dealing with fluoride, pregnancy, anaemia, low birth weight babies and the linkages to act upon for the benefit of maternal and reproductive child health programmes.

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