

## **APPENDIX C: TRANSLATION OF CHINESE/FETAL BONE STUDY**

*Zhonghua Yu Fang Yi Xue Za Zhi (Chinese Journal of Preventive Medicine).*  
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### **Relationship Between Bone Fluoride Content, Pathological Change in Bone of Aborted Fetuses and Maternal Fluoride Level [Article in Chinese]**

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**ABSTRACT:** Relationship between bone fluoride content, pathological change in bone of aborted fetuses and maternal fluoride level was studied in 46 pregnant women and their inducedly-aborted fetuses. Results showed fluoride content in fetal femur averaged 368.2 micrograms(ug)/g, and 41.4% of the bone with pathological change. Fluoride levels in maternal urine and amniotic fluid and fluoride content in fetal femur and pathological change in fetal femur appeared a positive correlation between them. Femur fluoride content and pathological change of bone in fetuses born to mothers with mottling teeth were significantly greater than to those without them. Pathological change in fetal femur presented dose-response relationship with their bone fluoride content. When the latter reached greater than 500 ug/g, pathological changes occurred in 90% of the bone.

Key words: Femur, Fluorine PMID: 7796679 [PubMed - indexed for MEDLINE]

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## **ENGLISH TRANSLATION**

### **Relationship of Maternal Fluoride Level**

There have been reports on how harmful fluoride is to human health. [1,2] It is known that fluoride can penetrate through the placenta sac and be stored in certain organs of the fetus. [3-5] The results of the fluoride content level in main organs of fetuses, especially, the bone fluoride content, can vary due to the limit of experimental conditions, methods, and lack of studies.[6.7] The following study shows the relationship between bone fluoride content and pathological change and maternal fluoride level. The fluoride content level in fetuses were measured and evaluated to determine if it was harmful to the bones.

### **Targeted Group and Research Method**

1. Targeted group: 46 women age between 20 and 40 who have been pregnant for at least 16 weeks and then had an abortion. These women and their fetuses were evaluated. Location: Au Han Qi, Mongolia autonomous region, China. A physical exam was conducted and the women were diagnosed as having the third level fluoride teeth stain (i.e. dental fluorosis). [8]

## 2. Method of collecting samples and examination:

### A. Fetus bone:

1) 0.5 cm mid segment of fetal femur was selected right after the abortion;

It was cleaned, dried, ground [9] and heated in a lab tube under 1050 degrees C for 25 minutes. Fluoride level was measured using a Fluoride Electronic Meter. The formula  $F \text{ microgram/g} = (\text{micromol/LX100})/W$  was used to calculate the fluoride level.

2) 0.2 cm from the same bone was selected. After being isolated and treated, it was viewed under a microscope to determine if there was a pathological change.

### B. Pregnant mothers' urine and amniotic fluid:

30 ml of urine was collected right after the pregnant mothers were admitted in the hospital. 5 ml of amniotic fluid was drawn right after abortion medicine was injected. The amniotic fluid was kept cool at a low temperature. The fluoride level was measured using a Fluoride Electronic Meter

3. Determination of fetus bone pathological change: Any one of the following conditions was considered to be a pathological change:

A. Disorder and uneven arrangement of the femur.

B. Uneven formation of internal bone cells (e.g. spongy bone) with empty spots (cavities) in the internal structure of the bone.

C. Enlargement of the blood vessels with increase of bone cells which later become bone (ossifying cells) and fiber tissue.

D. Even spread of the bone cells and decrease of the bone density.

4. Method of calculation and analysis: All data was collected by T/W computer system and analyzed by the Epi Info. Software.

## Results

Fetus fluoride content (level) in femur: The 46 fetuses were divided into 3 groups with an average femur fluoride content of 368.2 ug/g. There was no significant difference of the bone fluoride content in different fetuses at different months.

**Table 1 Average of fluoride content in fetuses in high fluoride areas**

Month of fetus	Number	Bone fluoride content (ug/g)
~ 4	16	399.3 ± 128.2
~ 6	9	346.7 ± 110.0
~ 7	21	353.8 ± 128.6
Total	46	368.2 ± 123.6

*F = 0.85, P>0.05*

Maternal fluoride level and fetus femur fluoride level and pathological change: The following Table 2 indicates that as the fluoride level increased in the mother's urine, there is an increase of bone fluoride level in the fetus. Statistical analysis showed there was a significant change. Obviously, the increase of fetus bone pathological change was affected by the increase of the level of urine and bone fluoride. There is a significant difference among the groups.

**Table 2 The level of maternal urine fluoride and the fetus femur fluoride and pathological change**

Urine fluoride (mg/L)	Number	Femur fluoride (ug/g)	Bone pathological change Number (Rate %)
<2.0	17	319.3 + 125.6	2 (11.8)
2.0	11	386.5 + 90.0	5 (45.5)
>4.0	18	403.2 + 126.4	12 (66.7)

*F=3.90, P<0.05 X<sup>2</sup>=10.97, P<0.01*

Fluoride level of amniotic fluid and fetus bone fluoride level and pathological change:

Changes are significant on the level of fetus femur fluoride and bone pathology along with the increase of the fluoride level in amniotic fluid (Table 3).

**Table 3 The level of maternal amniotic fluid and the level of fetus femur fluoride and pathological change**

Fluoride in AF (mg/L)	Number	Bone fluoride	Bone pathological change Number (Rate %)
<0.020	7	288.6 + 60.5	1 (14.3)
0.02	29	358.4 + 126.3	10 (34.5)
>0.070	10	452.4 + 111.2	8 (80.0)

*F = 4.37, P < 0.05, X<sup>2</sup> = 8.84, P < 0.05*

See Table 4 for the relationship between whether or not the mothers have fluoride teeth stain and fetus femur fluoride level and pathological change. There is a significant correlation between the two.

**Table 4. Whether or not mothers have fluoride teeth and the level of fetus bone fluoride and pathological change**

Mother's fluoride teeth stain	Number	Bone fluoride	Bone pathological change Number (Rate %)
Have	25	431.0 + 110.9	15 (60.0)
Don't Have	20	289.8 + 96.9	4 (20.0)

*t = 13.78, P < 0.01, X<sup>2</sup> = 7.29, P < 0.01*

The degree of mothers' fluoride teeth stain and the level of fetus femur fluoride and pathological change: divided 25 mothers with fluoride teeth stain into two groups based on the degree of fluoride in their teeth. The results showed there was no significant correlation change between the two ( $P > 0.05$ ).

Fetus femur fluoride level and bone pathological change: 3 groups were tested based on the fetus femur fluoride level. The results showed that there was a significant correlation change between the two, especially when the bone fluoride level is more than 500 ug/g, the pathological change rate can reach up to 90%, or 4.1. The correlation change is significant between the two ( $P < 0.01$ ).

#### **Discussion:**

Research done in the past indicate the highest bone fluoride level can be 1000 ug/g while the lowest can be 100-200 ug/g.[6] The main cause of this significant difference is that fluoride intake level affects the bone fluoride level, e.g. the more fluoride intake, the more bone fluoride level. The results of the average bone fluoride level found in this research is 368.2ug/g, is slightly higher than Mr. He Han's research results. This may be caused by sample and experimental differences.

The research results showed that there was a positive correlation between fetus femur fluoride level and the fluoride level in mothers' urine and amniotic fluid. This indicates, the high fluoride level in the body of mothers, the high fluoride level in fetus. The placenta sac barrier does not prevent fluoride buildup in the fetus. The research did not show any difference of fluoride levels in different months of the fetuses. This may due to a saturated condition when the fetuses are exposed to a high fluoride level that is stored in the bones. As the fetus increased in age each month, more fluoride went into the fetus bones and later reached an equilibrium or saturation point. Therefore, the discharge of fluoride also increased. Another possible explanation is the small sample of fetuses involved.

Another finding from the research is that there is a strong relationship between if mothers have fluoride teeth and the fetus bone fluoride level and pathological change. It is acknowledged that the fluoride teeth only reflect individuals who had an exposure to certain degree of fluoride in the past. It does not reflect the current intake or exposure to fluoride and the fluoride level in mother's body. Therefore, this aspect should be further investigated with additional research.

## **Conclusion:**

In conclusion, there is a relationship between fluoride level in maternal urine and amniotic fluid and the fetus bone fluoride level and femur pathological change. This study proves that fluoride buildup in fetuses does cause harm to the bones of fetuses. The degree of damage can be evaluated by testing the fluoride level in maternal urine. Another finding that needs be emphasized is that when the fetus femur fluoride level is greater than 500ug/g, pathological changes occurred in 90% of the bone. The high fluoride level in the fetus resulted from the high maternal fluoride level. These are mothers who live in high fluoride risk areas. Prevention and treatments are essential to these people.

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