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Morphological and cytogenetic effects of hydantoin and primidone. Experimental study

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Abstract

In the light of accumulated observations on the effects of hydantoin and primidone, the authors wish to identify their effects on A2G mice fetus karyotype and to establish possible specific aspects of the macro- and microscopical morphology of the foetal malformed structures due to hydantoin and primidone exposure during early pregnancy.

As experimental model we used six different groups according to the international rules of animal care: 3 A2G mice control groups; 2 hydantoin groups, where H1 A2G mice were i.v. injected with 25mg hydantoin day 1-5 of gestation,./100g.body weight.and H2 25mg. /100g body weight p.o.; 1 primidone group that received 10,000 ppm primidone (equivalent to average daily doses of approximately 970 mg primidone/kg body weight in humans). Parametric data including number of live fetuses/maternal unit, body weight, abnormalities, were evaluated. The Cytogenetic study of the maternal-foetal unit was performed in each case; cariotypes were performed for 1 female and 2 of their foetuses in each litter, analysing 50 meiosis for each. Statistical comparison were performed between the control group and treated groups by student's unpaired t-test; the level of significance chosen for all results was $t<0.05$.

The results reveal morphological landmarks of intra-uterine growth retardation as decreased length and weight, micrognatia, microcephalia, micromelia of the hydantoin groups. Decreased body weight of the maternal-foetal unit and decreased live-fetuses number were observed following early neonate exposure to the hydantoin ($t<0,05$). The malformed tissues of the phenytoin groups has peculiar microscopic aspects as vascular ecstasy, vascular ecotypes, aglutinated red blood cells and some time hematoma which was in a position to inhibit the normal growth of the tissue in the phenytoin groups.

The obtained data allow the authors to conclude that hydantoin and primidone treatment in the early gestational period may modify the normal development process and decrease the maternal-foetal developments' rate while induce general development retardation, abnormal morphogenesis and peculiar aspects of the chromosomes (rearrangement, ring chromosome, centric atraction of the chromosomes).

Keywords: hydantoin, primidone, maternal-foetal unit, developpement rate, mice, ring chromosome, chromosome rearrangement.

Introduction

Hydantoin is a major anticonvulsant drug that is very effective in controlling a wide variety of seizure disorders while impairing neurological function little, if at all (LO & FRIEDMAN, [1]. Early work suggested the hypothesis that the drug's effects were due to a selective block of high-frequency neuronal activity. The effects of hydantoin can be explained by a use- and frequency-dependent suppression of the sodium action potential by hydantoin, with a consequent filtering out of sustained high-frequency neuronal discharges and synaptic activity. The molecular mechanism for this is a voltage-dependent blockade of membrane

sodium channels responsible for the action potential (HOLMES LB & al., [2]. Through this action, hydantoin obstructs the positive feedback that underlies the development of maximal seizure activity, while normal brain activity, proceeding at lower neuronal firing rates, is spared its depressant action.

The initial descriptions of the phenytoin teratogenicity were published in the early 1970s (LOUGHNAN & al, [3]). In 1966, MASSEY [4] reported the teratogenic effect of phenytoin in mice (MASSEY, [4]). HANSON & SMITH coined the term "fetal hydantoin syndrome" (HANSON & SMITH, [5]). Many of those early reports (HOLMES & al, [2]) were clouded by the in utero exposure to other antiepileptic drugs. The main and most constant anomalies seen in patients exposed to hydantoine during the pregnancy is growth deficiency (BUEHLER & al, [6]). Cleft lip and palate, developmental delay, mental retardation, and mid-face hypoplasia are also supportive of the diagnosis (ARTAMA, [7]). Exposure of the embryos to the action of hydantoin decreased embryo's survival rate and weight and induce neural development retardation (NODEN, [8]) and abnormal morphogenesis (also have been reported by Ogura 2002 [9]). Primidone exposure in utero has been implicated in the development of specific facial dysmorphology. Cleft lip and palate have also been reported (ROSA, [10]).

In the light of accumulated observations (NODEN, [8], ARTAMA, [7]) on the effects of hydantoin and primidone, the authors wish to identify their effects on A2G mice fetus karyotype and to establish possible specific aspects of the macro- and microscopical morphology of the foetal malformed structures due to hydantoin and primidone exposure during early pregnancy.

Material and Methods

Female A2G mice were kept in a vivarium with a controlled 12 hours lights regimen and were fed with the same diet. They were always three females to one male and the first day of gestation was the day when the vaginal plug was founded. The basic sample unit was the litter. As experimental model we used three different strains according to the international rules of animal care: A2G mice as control groups, A2G mice as hydantoin experimental groups H1, H2 and A2G mice as primidon experimental group P. Three pregnant females formed each of the control groups C1, C2, C3. The control group C1 were kept in similar conditions with the H1 hydantoin group, the control group C2 in similar conditions with the H2 hydantoin group including the i.v. punction, the control group C3 in similar conditions with the P primidone group. The H1 group consisting of three female were given phenytoin suspended in 1% carboxymethyl cellulose solution. Since the blood concentration of phenytoin depends on the size of the phenytoin particles, the powdered hydantoin was filtered with 200 mesh (stainless steel, pore size 74). At the H1 group the dose of hydantoin was set at 25mg. /100g body weight p.o. Daily to 8 o'clock during first 10 days of gestation. The H2 pregnant mice groups were hydantoin i.v. injected 25mg./100g.body weight, daily to 8 o'clock during first the 10^{th} – 12^{th} days of gestation. The P primidone group received 10,000 ppm primidone (equivalent to average daily doses of approximately 970 mg primidone/kg body weight in humans). The primidone was administered orally using adequate dilutions of a commercial formulation (Liskantin®-Saft, Desitin-Werk Carl Klinke, Hamburg). The volume administered was 5 ml/kg. At the time of the experiment, the pregnant mice in the first day of pregnancy had an average weight of 41 ±3 g.

In the present study the administration of teratogen agents covered the organogenesis phase. At 12 days gestation age the females were killed (by the cervical dislocation) and the embryos were removed from the uterus, weighed and divided into weight classes with 25 mg.

intervals. Parametric data including number of live fetuses/maternal unit, body weight, congenital abnormalities, were evaluated in hydantoin, primidon and control groups. The morphological data obtained using the necropsy technique were synthesised as in the table NR. 1. Statistical comparison were performed between the control groups, hydantoin and primidon groups by student's unpaired t-test; the level of significance chosen for all results was $t<0.05$..

The Cytogenetic study of the maternal-foetal unit was performed in each case; cariotypes were performed for 1 female and 2 of their foetuses in each litter. We analysed 50 meiosis in each case, and the results were statistically evaluated by the t- student test.

Table 1. General data of A2G mice necropsy

Experiment NR.	Date	Sex	Strain	Histology	Photo
animal	Body Weight	Treatment	Account	Exp. Observation	
THE ORGAN		T* Trimmed		P** Pathologic	
Skin					
Mammary Tissue					
Lymph nodes					
Ovaries					
Bulbourethral Gland					
Uterus / Cervix / Preputial Gland					
Bladder/ urethra					
Testes/ Epididymes					
Prostate/ seminal vesicles					
Stomach					
Intestines					
Cecum/colon					
Spleen					
Pancreas					
Liver					
Kidney					
Adrenal					
Heart					
Thymus					
Lungs					
Thyroids					
Skull/nasal passages/					
Oral cavity					
Brain					
Pituitary gland					
Spinal column/ Spinal cord					
Other Tissue					

Results

The study of the maternal-foetal units' development rate reveals that hydantoin and primidone modify the normal development process, significant statistic increase the mortality rate and the number of the autolised fetus (table 1).

The three A2G mouse materno-foetal units of the H1 hydantoin group have 31 live fetus as following: 7, 9, 15 with the medium number of fetus 11.0±4.0.

The three A2G mouse materno-foetal units of the H2 hydantoin group have 26 live fetus as following: 6, 8, 12 with the medium number of fetus 9.0± 3.0.

The three P primidone materno-foetal units had 19 live fetus as following: 5, 7, 7 with the medium number of fetus 6.0± 1.0. The first primidone materno-fetal unit was 5 live fetus and 3 autolised, the second primidone materno-fetal unit was 7 live and 5 dead autolised fetus; the third primidone materno-fetal unit was 7 live and 4 dead autolised fetus.

The hydantoin and primidone are capable of inducing significant statistic decreased number of live foetuses, compared to the control groups (Table 2.).

Table 2. Foetal mortality in control groups, hydantoin and primidon treated mice

	A2G pregnant mice Nr	Foetal mortality / litter (%)	Live foetus/ litter total NR	Live foetus/ litter Medium± SD	Live foetus /litter Minim NR	Live foetus/ litter Maxim NR	t test
C1	3	10± 4.9	40	14.0±3.0	11	17	
C2	3	10.6±5.5	41	14.0±1.0	13	15	
C3	3	10.1±4.1	44	15.0±2.0	13	17	
H1	3	13.1±4.6	31	11.0±4.0	7	15	0.031612**
H2	3	15.2±5.3	26	9.0± 3.0	6	12	0.043225**
P	3	38.4±1.7	19	6.0± 1.0	5	7	0.042312**

t* where student test mean: t<0.05
t** where student test mean: t<0.005

The live foetuses' weight is a reflection of the significant intra-uterine growth retardation of the hydantoin and primidone groups (table 2).

Table 3. Foetal weight in control groups, hydantoin and primidone treated mice

	Foetal mortality/ litter (%)	Foetal weight/ litter (g) mead±SD	Foetal weight/ litter Minim weight	Foetal weight/ litter Maxim weight	t test
C1	10± 4.9	1.83± 0.10	1.73	1.94	
C 2	10.6±5.5	1.94± 0.05	1.89	2.00	
C 3	10.1±4.1	1.68± 0.05	1.63	1.73	
H1	13.1±4.6	1.03± 0.15	1.08	1.18	0.000809**
H2	15.2±5.3	1.16± 0.07	1.09	1.23	0.000796**
(P)	38.4±1.7	1.16± 0.10	1.06	1.26	0.000778**

The morphology study of the hydantoin group reveals micrognatia, microcephalia, cleft palate, cleft lip, hypoplasy of the cerebral hemispheres. The malformed tissues of the hydantoin groups has peculiar microscopic aspects as vascular ecstasy, vascular ectopyes, aglutinated red blood cells and some time hematoma which was in a position to inhibit the normal growth of the tissue in the hydantoin groups.

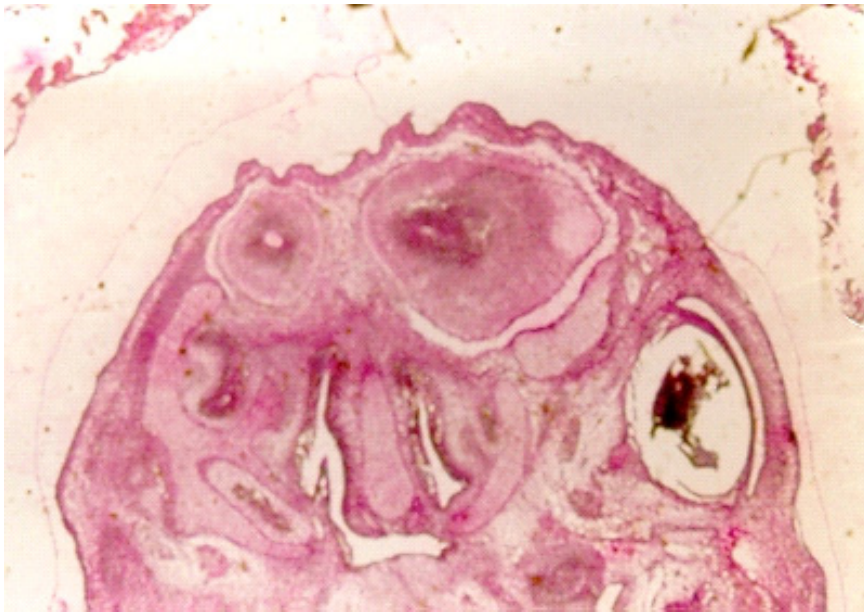

Figure 1. A2G mouse foetus day 14, hydantoin group, hypoplasy of the right cerebral hemisphere and hypoplasy of the right eye. HE. Ob.X 6.

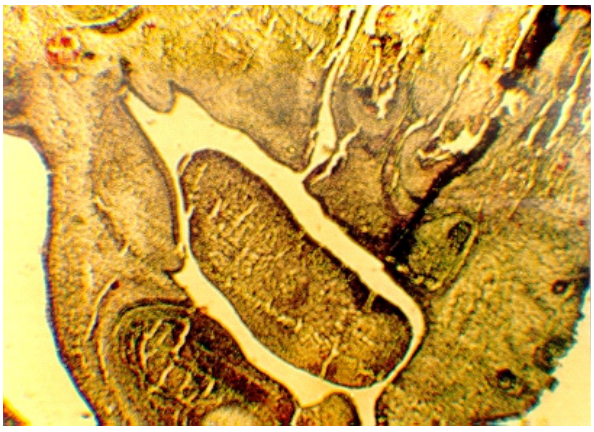

Figure 2. A2G mouse foetus day 14, hydantoin group. Microcephalia, right unilateral cleft lip, cleft palate, left unilateral anophtalmie. Ob.X 6. HE

Microscope exam of the nasal septum from the same case shows vascular ectopy and sludge hematies; necrosis of the cartilaginous septum, passive hyperaemia and sludge hematies.

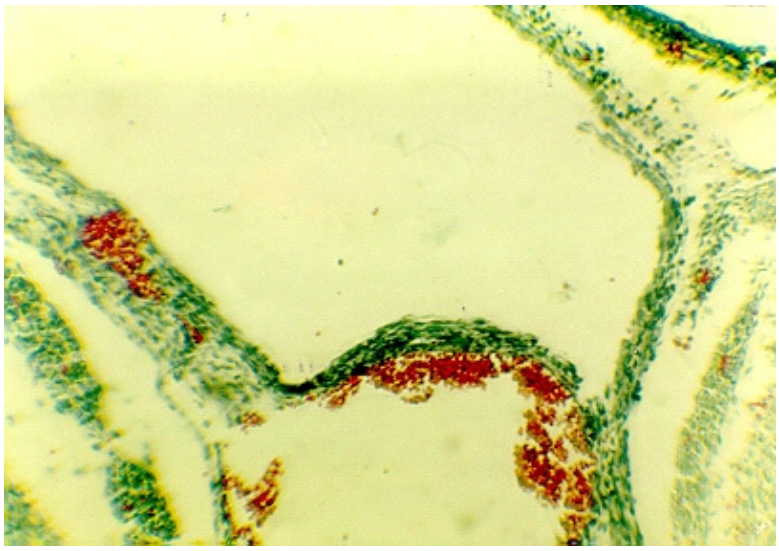

Figure 3. A2G . mouse foetus day 14, hydantoin group, Microscope exam of the palate tissue shows necrosis of the chondroid tissue, vascular ecstasy, increased wall of the small veins, lymph tissue with passive hyperaemia and sludge hematies HE Ob.X 40.

The morphology study of the primidone group shows cephalic extremity abnormalities and a large number of autolised foetuses.

Histology study of primidone group has similar aspects with hydantoin group: vascular ecstasy, haemorrhage and necrosis in the facial area.

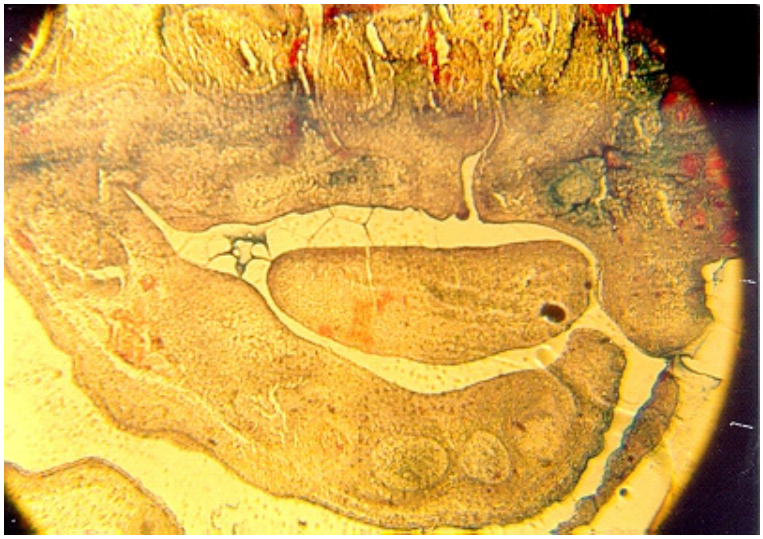

Figure 4. A2G mouse foetus day 14, primidone group, left unilateral cleft lip, cleft palate. H.E.Ob.X 6.

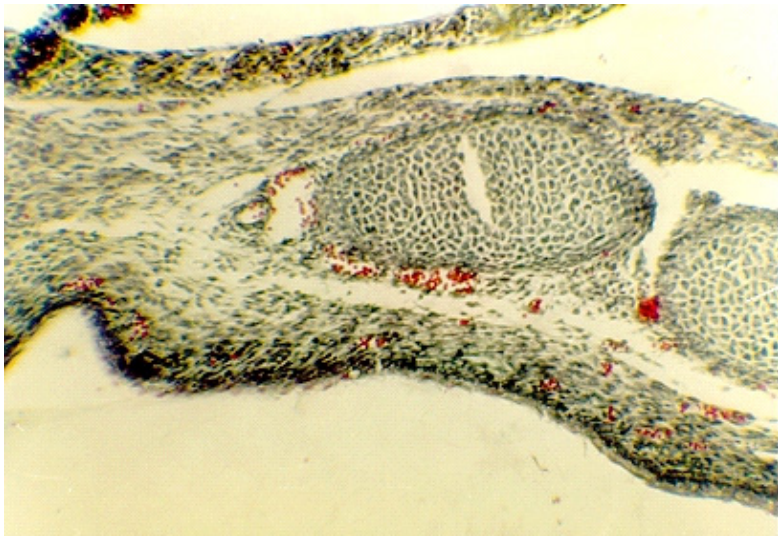

Figure 5. A2G mouse foetus day 14, primidone group, fetus'maxilla shows necrosis of the cartilaginous tissue, ulcer of the cubic tissue and sludge hematies in the capillaries, vascular ecstasy, increased wall of the small veins, lymph tissue with passive hyperaemia and sludge hematies. HE Ob.X 40.

The results of the cytogenetic study indicate very large number of embryos with abnormal metaphases in primidone and hydantoin groups and the absence of the abnormal metaphases in the control groups, as in table 4.

Table 4. *Incidence of abnormal metaphases in control groups, hydantoin and primidone treated mice*

			normal metaphase/specimen NR.					abnormal metaphases/ specimen NR.				
Group	Studied mice NR	Studied meta-phases NR.	X min.	X max.	**X med**	S.D.	t- test	Xo	Xm	**X med**	S.D.	t - test
C1	2 pregnant A2Gmice	50	25.0	25.0	**25.0**	0.0	-	0.0	0.0	**0.0**	0.0	-
	2 foetuses	50	25.0	25.0	**25.0**	0.0	-	0.0	0.0	**0.0**	0.0	-
C2	2 pregnant A2Gmice	50	25.0	25.0	**25.0**	0	-	0.0	0.0	**0.0**	0.0	-
	2 foetuses	50	25.0	25.0	**25.0**	0	-	0.0	0.0	**0.0**	0.0	-
C3	2 pregnant A2Gmice	50	24.0	25.0	**24.5**	0,5	0,08	0.0	1.0	**0.5**	0.5	0,08
	4 foetuses	100	23.0	25.0	**24.0**	1.0	0,107	0.0	2.0	**1.0**	1.0	0.104
H1	2 pregnant A2G mice	50	20.0	24.0	**22.0**	2.0	0,001	2.5	3.5	**3.0**	0.5	0.001
	4 foetuses	100	18.0	20.0	**19.0**	1.0	0,001	5.0	7.0	**6.0**	1.0	0.001
H2	2 pregnant A2G mice	50	19.0	23.0	**21.0**	2.0	0,001	3.0	5.0	**4.0**	1.0	0.001
	4 foetuses	100	16.5	19,5	**18.0**	1,5	0,001	6.0	8.0	**7.0**	1.0	0.001
P	2 pregnant A2G mice	50	11.0	14.0	**13.0**	1.0	0,001	11.0	13.0	**12.0**	1.0	0.003
	4 foetuses	100	5.0	7.0	**6.0**	1.0	0,003	18.0	20.0	**19.0**	1.0	0.003

The phenytoin group and the primidone group have statistic significant abnormal metaphases comparative to control group ($t<0.005$). The hydantoin H1 group has 6.0

abnormal metaphases (medium number) for each 25 examined metaphase/ A2G foetus. The hydantoin H2 group has 7 abnormal metaphases (medium number) for each 25 examined metaphases/A2G foetus. The H1 and H2 hydantoin groups have chromosomes' abnormalities especially at 2 chromosome pairs and chromosomal rearrangement (Figure NR 6, Figure NR.7).

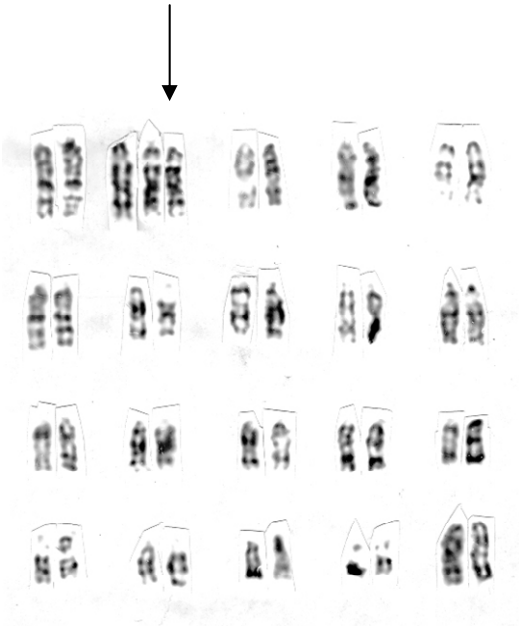

Figure 6. H2 Hydantoin group, facial malformed A2G mice foetus, left anophtalmy.
Karyotype 41,XX, +2 (arrow)

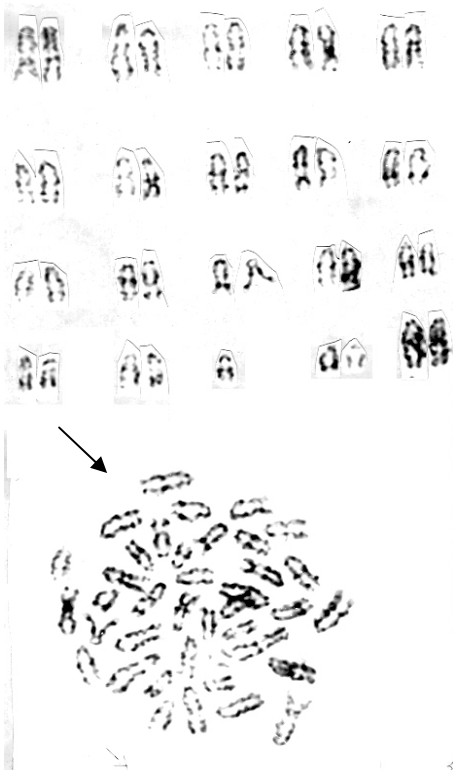

Figure 7. H1 Hydantoin A2G mice foetus with meningocel and right anophtalmy
Karyotype 40,XX,
Chromosomal rearrangement (arrow)

The primidone group has statistic significant abnormal metaphases comparative to the control group ($t<0.005$), with 19.0 medium number of abnormal metaphases for each 25 examined metaphases/A2G foetus'mice and 2 abnormal metaphases for each 25 examined metaphase/pregnant A2G mice. Primidone group has peculiar aspects as ring chromosome and central attraction (Figure NR 8, Figure NR.9).

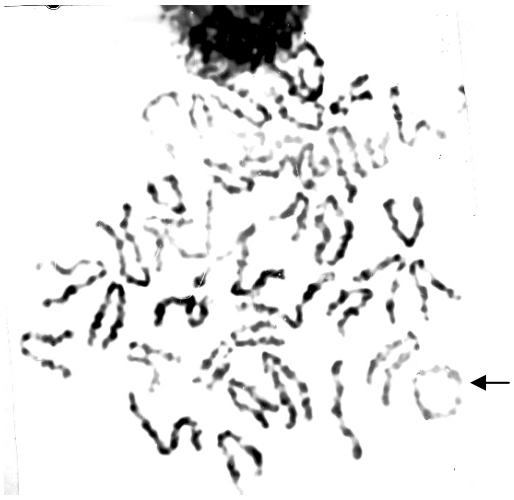

Figure 8. Primidone group, A2G mice foetus with cleft palate
Karyotype 40, XY
Ring chromosome (arrow)

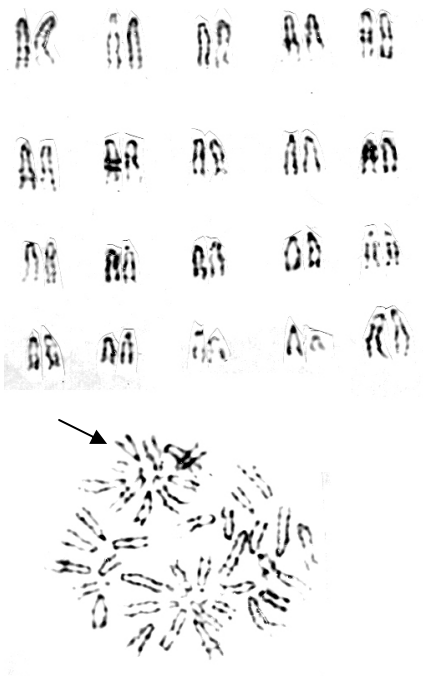

Figure 9. Primidon group, A2G mice foetus, cleft lip, cleft palate.
Karyotype 40,XY.
Central attraction (arrow)

Conclusions

Decreased of the maternal-foetal unit and decreased live-fetuses number were observed following early neonate exposure to the hydantoin and primidone.

The decreased number of ive foetuses of the hydantoin groups associates decreased body weight of the live fetus and significant morphological landmarks of the intra-uterine growth retardation as decreased body-weight.

The action of the phenytoin and primidon modify the normal development process and induce general development retardation (hydantoin), necrosis (primidone) and abnormal morphogenesis (hydantoin and primidone).

Intake of the hydantoin and primidon during embriogrenesis periode may generate peculiar aspects of the chromosomes (rearrangement, ring chromosome, central atraction of the chromosomes) but this finding need to be continued with semnificativ statistic number litters.

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