TEL AVIV UNIVERSITY

Non-teratogenic environmental toxicant exposure of organogenesis-stage embryos results in osteoporotic bones in adults

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By now, epidemiological observations and experimental studies strongly suggest that inadequate maternal nutrition during pregnancy can predispose to age-related osteoporosis

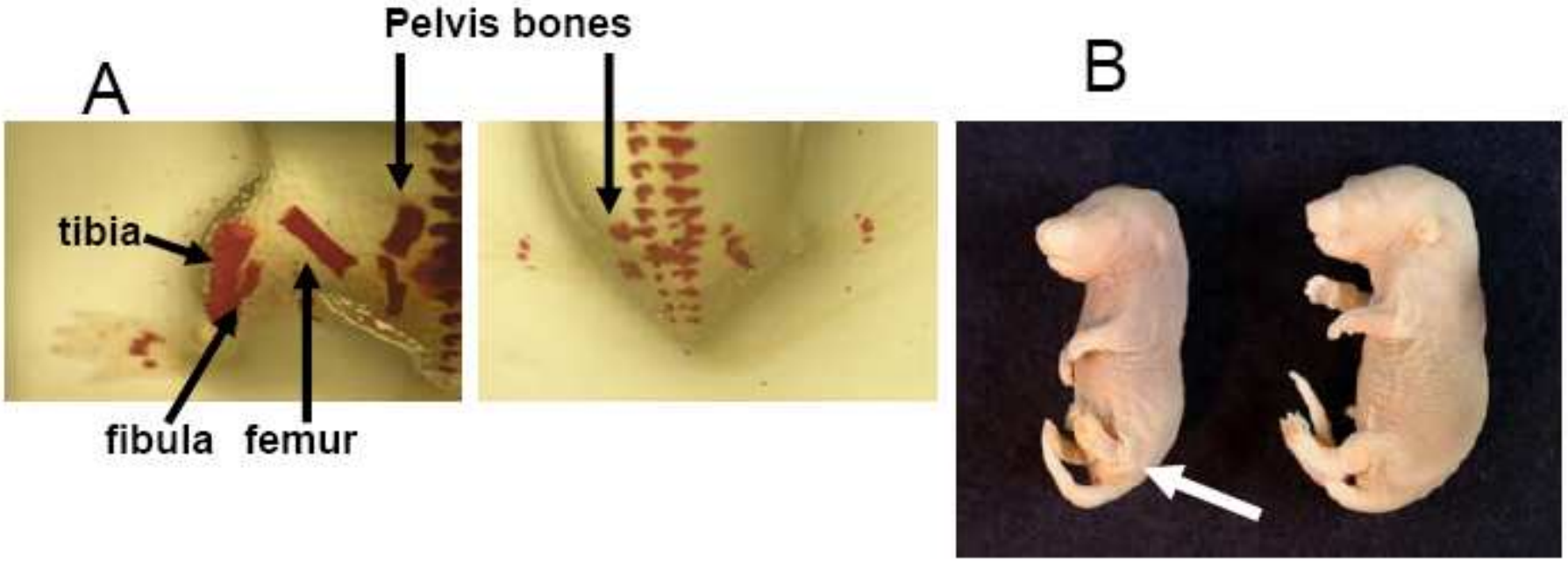
Yet, nutrient imbalances associated with osteoporosis also affect

- maternal endocrine homeostasis
- prenatal skeletal development
- fetal growth and growth trajectory of offspring

We asked
whether in utero exposure to an environmental chemical
at a dose inducing neither maternal toxicity nor structural or
functional developmental abnormalities can result in
the appearance of osteoporotic bones in adult offspring.

We chose to explore this possibility
in an experimental study in mice exposed to
5-aza-2-deoxycytidine (5-AZA)

Being injected on day 10 of gestation, 5-AZA induces phocomelia (absence of limb long bones) of the hindlimbs in a dose-dependent fashion

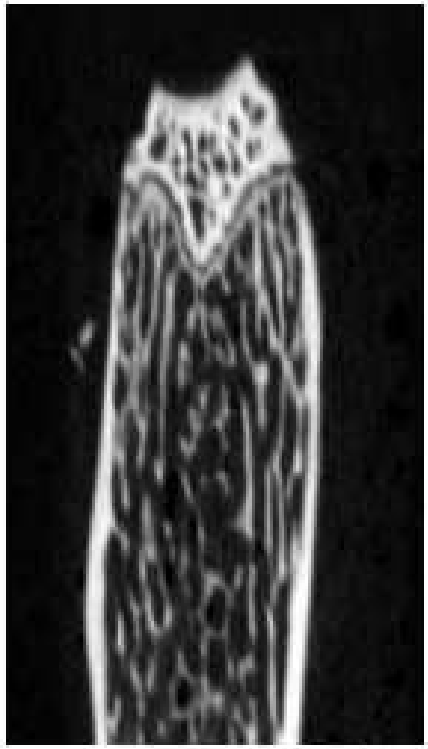


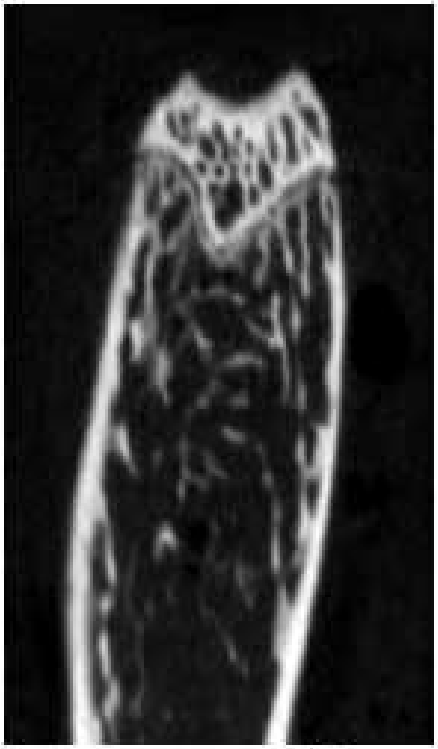
In our experiments in ICR mice

Dose mg/kg	Number of tested litters	Implantation sites/litter (M ± SE)	Postimplan-tation loss (resorptions) (arcsine, M ± SE)	Fetal weight (g) (M ± SE)	Fetuses exhibiting phocomelia (%)
control	8	10.3 ± 0.6	7.3 % (16.2±2.6) [a]	1.28 ± 0.05 [a]	0
0.75	8	10.6 ± 0.6	71.8% (57.7±3.8) [b]	0.80 ± 0.04 [b]	100
0.50	8	11.4 ± 0.4	13.2% (21.6±4.2) [a]	0.98 ± 0.05 [c]	100
0.25	12	11.0 ± 0.4	10.6% (19.3±2.2) [a]	1.10 ± 0.07 [c]	74.6
0.15	12	10.8 ± 0.7	9.2% (18.0±2.9) [a]	1.34 ± 0.04 [a]	0

0.15 mg/kg of 5AZA was considered as a sub-threshold teratogenic dose

We exposed pregnant females to 0.15 mg/kg 5-AZA and then femora of 5-month old male offspring were scanned by micro-CT



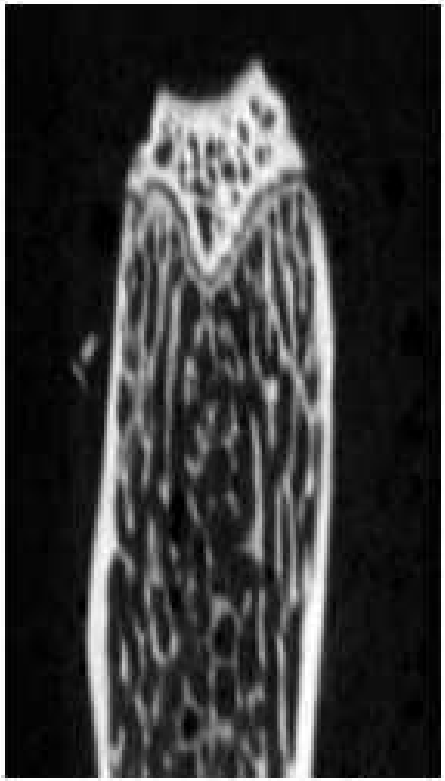
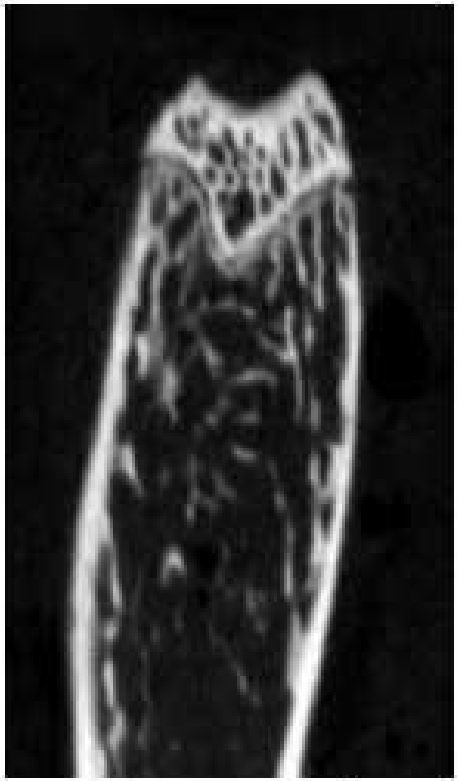
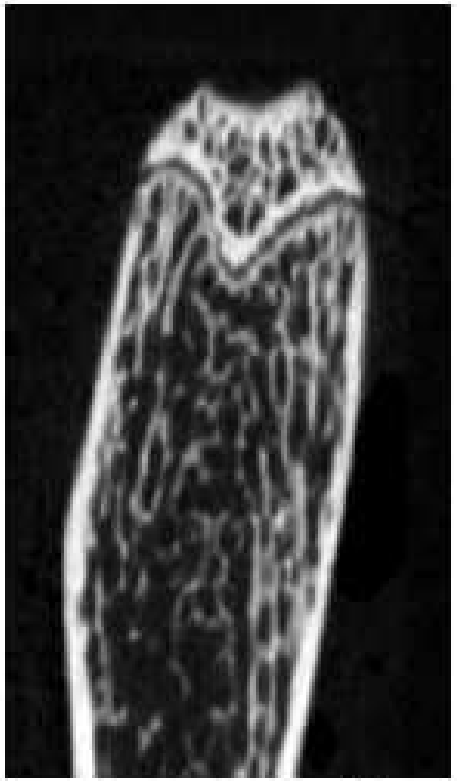


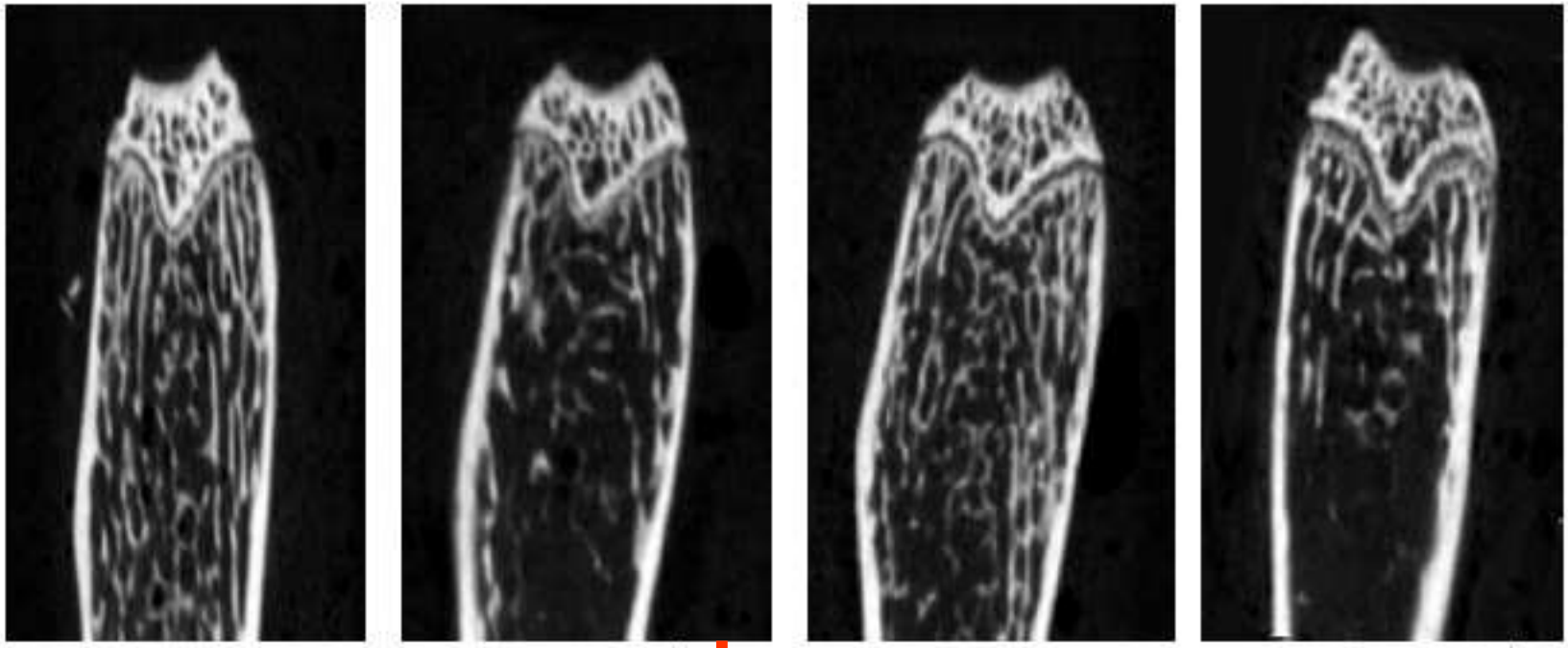
	Control	5-AZA
% Bone volume	32.601 ± 3.32[a]	21.720 ± 3.32[b]
Trabecular separation	0.169 ± 0.034[a]	0.205 ± 0.034[b]
Trabecular number	3.925 ± 0.428[a]	2.765 ± 0.428[b]
BMD	1.171 ± 0.010[a]	1.157 ± 0.010[ab]

The exposure of pregnant females to 5-AZA destroyed femoral microarchitecture in offspring

We also asked
whether exposure of embryos to environmental chemicals
can result in the altered sensitivity of adults to the lifestyle factors
implicated in the etiology of osteoporosis

Three-month old offspring were subjected every week, over a period of 5 weeks, to chronic mild stress (CMS) that has been shown to induce bone loss in mice (Yirmiya et al. 2006) .

	Control	5-AZA	CMS
			
% Bone volume	32.601 ± 3.32^{a}	21.720 ± 3.32^{b}	33.171 ± 3.32^{a}
Trabecular separation	0.169 ± 0.034^{a}	0.205 ± 0.034^{b}	0.150 ± 0.034^{a}
Trabecular number	3.925 ± 0.428^{a}	2.765 ± 0.428^{b}	4.146 ± 0.428^{a}
BMD	1.171 ± 0.010^{a}	1.157 ± 0.010^{ab}	1.149 ± 0.010^{bc}



	Control	5-AZA	CMS	5-AZA+CMS
% Bone volume	32.601 ± 3.32^{a}	21.720 ± 3.32^{b}	33.171 ± 3.32^{a}	18.523 ± 3.32^{b}
Trabecular separation	0.169 ± 0.034^{a}	0.205 ± 0.034^{b}	0.150 ± 0.034^{a}	0.284 ± 0.034^{c}
Trabecular number	3.925 ± 0.428^{a}	2.765 ± 0.428^{b}	4.146 ± 0.428^{a}	2.327 ± 0.428^{b}
BMD	1.171 ± 0.010^{a}	1.157 ± 0.010^{ab}	1.149 ± 0.010^{bc}	1.135 ± 0.010^{c}

The exposure to 5-AZA increased the potential of CMS to induce skeletal deficiency

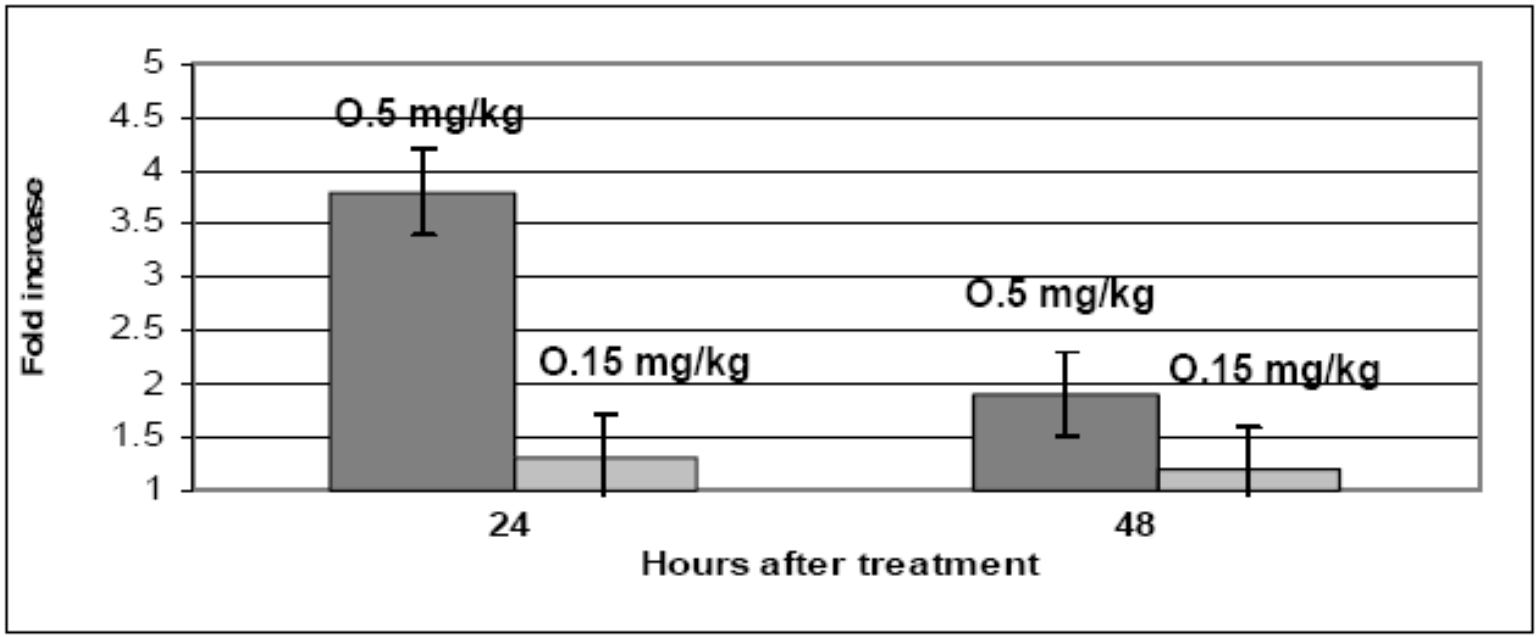
The results of studies addressing the pharmacokinetics and toxicity of 5-AZA suggested that 5-AZA induced bone loss in offspring by targeting the embryo but not the female

Excessive apoptotic cell death has been implicated in the pathogenesis of 5-AZA induced phocomelia

As 5-AZA was injected in the very beginning of osteoblastogenesis

the possibility that reduced osteoblast number might be responsible for 5-AZA–induced bone loss in offspring seemed conceivable

We checked this possibility by evaluating caspase 3 activity in hind limb buds



These results indicate excessive apoptotic cell death as a pathogenetic event for 5-AZA teratogenesis but not for 5-AZA-induced bone loss in adult offspring.

We then performed a global analysis of microRNA expression in hind limb buds of embryos harvested 24 hours after 5-AZA injection

MicroRNAs (miRs) are non-coding RNAs that attenuate gene activity posttranscriptionally by inhibiting effective mRNA translation of target genes

We observed that 5-AZA treatment increases the expression of 18 microRNAs (miR) including *miR-135b* and *miR-200a*,

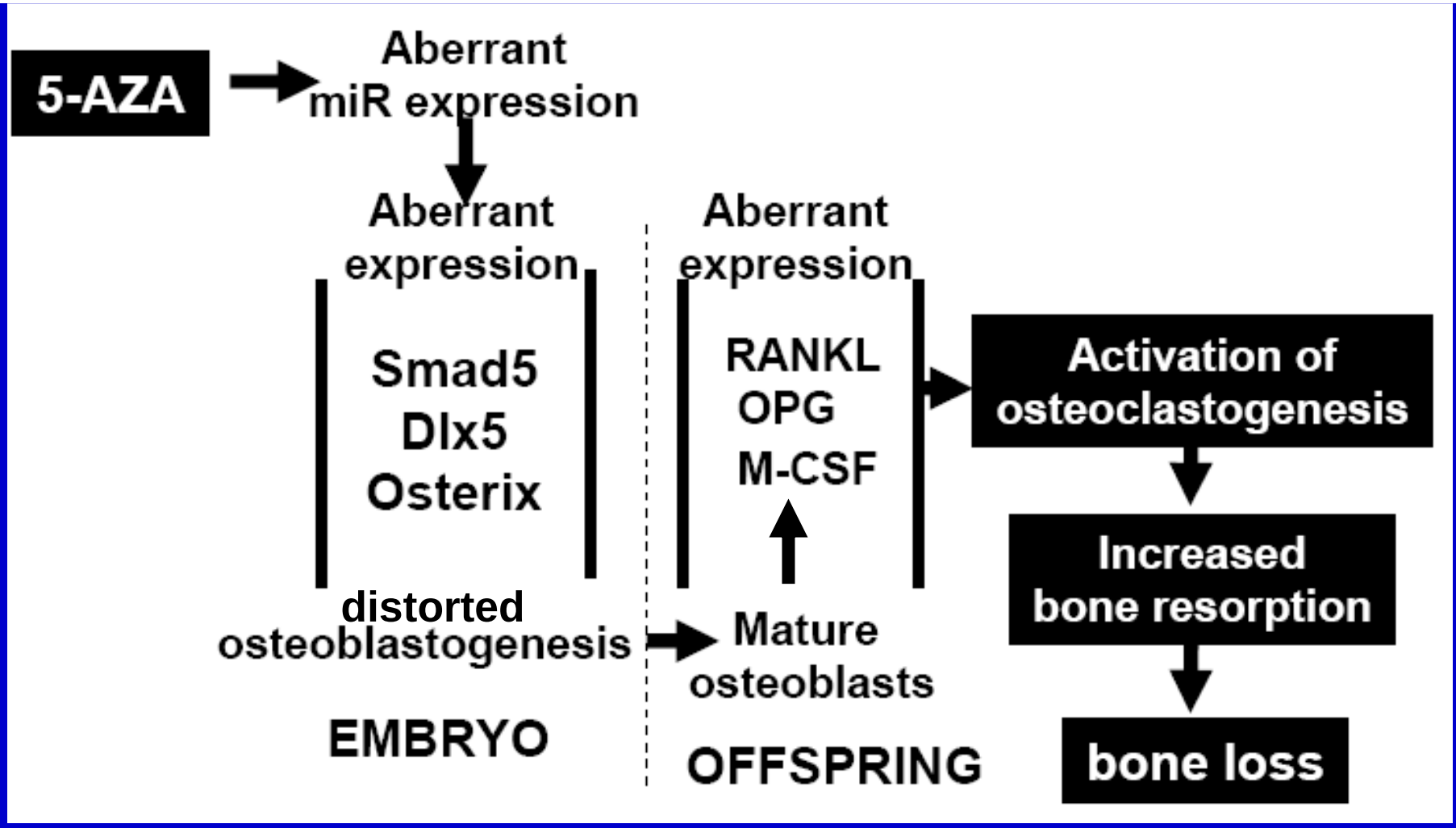
miR-135b has been shown to regulate the expression of *Smad5,* the intracellular BMP2 receptor for osteoblast differentiation

and

Osterix which acts downstream of a global regulator of osteoblastogenesis, the runt-related transcription factor 2, to induce mature osteoblasts

miR-200a has been shown to regulate the expression of *the distal-less homeobox 5 (Dlx5),* a transcription factor, which is expressed at the early stages of osteoblast differentiation

Based on the above observations, we tried to hypothesize the main steps of the pathway which 5-AZA can engage to induce bone loss in offspring



5-AZA-induced modulation of miRs expression might affect embryonic osteoblastogenesis thus leading to the appearance of mature osteoclasts unable to properly regulate postnatal bone remodeling

CONCLUSION

This study demonstrates that a single exposure of pregnant females to environmental chemicals at a dose level inducing neither maternal toxicity nor anatomical or functional developmental abnormalities may increase the risk of osteoporosis

Studies with other environmental chemicals are warranted because their results may have a fundamental impact on our understanding of the etiology and pathogenesis of OP and our developmental toxicity testing strategies.