



## Environmental Impact Analysis on Chicken Embryo Development: A Systematic Review

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**Abstract.** Environmental stress can affect the development of the embryo, including causing the death of the embryo. Environmental factors that can cause stress include salinity and zinc sulfate, zinc sulphate is a trace element and can affect embryonic development. The purpose of this study was to analyze the environment in Chicken embryonic development. A literature search was carried out systematically through the PubMed, NCBI, Google Scholar databases using keywords, namely, "heat, stress, embryo, chicken". Based on these keywords, the articles obtained were first selected by setting several inclusion criteria. Articles that do not meet the inclusion criteria are eliminated, and articles that meet the criteria will be analysed to obtain data. Based on the search results 21326 articles were obtained, and 21 articles that meet the inclusion criteria were selected. The result above also showed that heat stress give impact to development of embryo. From the results of the research conducted, it can be concluded that the environment has an impact on the development of the embryo. Further studies are needed, especially those related to other types of stress that can affect embryonic development in various types of animals.

**Keywords:** Analysis, Chicken, Embryo Development, Environment, Google Scholar.

### 1. BACKGROUND

A halt or interruption in embryonic development may occur as a result of harmful environmental factors. According to Effendi (2012) and Hutagalung et al. (2017), salinity is one environmental element that might put the embryo under stress (Effendi, 2012; Hutagalung et al., 2017). According to Mubarokah et al. (2014), one common method to speed up the hatching process is to manage the salinity. As a standard for egg hatchability, salinity measures the concentration of Na<sup>+</sup> and Cl<sup>+</sup> ions in water. Both the egg quality and the hatching time are affected by salinity. Salt affects the osmotic pressure that fish eggs experience. Fish eggs have an unstable osmotic pressure when exposed to salt levels that do not correspond to their pace of development (Hadid et al., 2014; Vallee & Auld, 1990). Therefore, a lot of energy is required to keep this pressure running. Isoosmotic salinity, which is close to osmotic pressure, was determined by Heltonika (2014) to be the optimal environment for embryonic development. This is particularly the case when thinking about how eggs stay alive while being incubated. Zinc (Zn) is an element that is present in all living things and is essential for the normal functioning of several enzymes. According to research conducted by Iguchi (1985), and Miller (1981), and Sandstrad and Evans (2000), zinc is an important component for over fifty enzymes to date (Iguchi & Sano, 1985; Miller & Nriagu, 1981; Sandstrad & Evans, 2000). Zinc

deficiency affects organogenesis, which in turn affects bone growth, according to many studies. Suprianti et al. (1983), Miller and Neathery (1981), and Tienhoven (1968) are among the research that fall within this category. The fundamental reason for doing this research was to find out how different environmental variables affected the growth of hen embryos (Miller & Nriagu, 1981; Suprianti et al., 1983; Tienhoven, 1968).

## **2. THEORETICAL STUDY**

Embryonic development is a highly regulated biological process that is sensitive to environmental conditions. Disruptions or arrests in embryogenesis may occur when embryos are exposed to unfavorable external factors, resulting in impaired growth, delayed development, or mortality. Among various environmental stressors, salinity plays a critical theoretical role in determining embryonic viability, particularly in oviparous organisms. Salinity reflects the concentration of sodium ( $\text{Na}^+$ ) and chloride ( $\text{Cl}^-$ ) ions in the surrounding medium and serves as an important physicochemical parameter influencing egg hatchability and developmental timing.

From a physiological perspective, salinity directly affects the osmotic pressure experienced by embryos. Developing eggs possess semi-permeable membranes that regulate water and ion exchange with their environment. When ambient salinity deviates from the embryo's developmental requirements, osmotic imbalance occurs, leading to physiological stress. Such conditions force embryos to expend substantial metabolic energy to maintain osmotic homeostasis, thereby diverting energy away from growth and differentiation processes. Prolonged exposure to non-optimal salinity can therefore impair embryonic development and reduce survival rates.

The concept of isoosmotic salinity provides a theoretical framework for understanding optimal embryonic environments. Isoosmotic conditions refer to salinity levels that closely match the internal osmotic pressure of the developing embryo. Under these conditions, minimal energy is required for osmoregulation, allowing metabolic resources to be allocated primarily toward cell division, differentiation, and organ formation. Consequently, isoosmotic salinity is theoretically considered the most favorable condition for embryonic development and egg survival during incubation.

In addition to physicochemical parameters, micronutrients play an essential theoretical role in embryogenesis. Zinc (Zn) is a trace element universally present in living organisms and is indispensable for normal cellular and biochemical functions. At the molecular level, zinc acts as a structural or catalytic cofactor for a wide range of enzymes involved in DNA synthesis,

protein metabolism, and cellular signaling. It has been identified as a critical component of more than fifty enzyme systems, underscoring its fundamental role in metabolic regulation.

Zinc availability is particularly important during embryonic development, as it influences organogenesis and skeletal formation. Zinc deficiency has been theoretically associated with disruptions in cell proliferation, impaired bone growth, and abnormal organ development. These effects arise because zinc-dependent enzymes are directly involved in processes such as nucleic acid synthesis, gene expression, and tissue differentiation. Therefore, adequate zinc levels are necessary to support normal embryonic growth and structural development.

Based on these theoretical considerations, embryonic development can be understood as the outcome of complex interactions between environmental factors, such as salinity, and essential micronutrients, such as zinc. Environmental conditions that optimize osmotic balance and nutrient availability are theoretically expected to enhance embryonic viability, growth, and successful hatching. This framework provides the conceptual basis for investigating how variations in environmental parameters influence the development of hen embryos.

### **3. RESEARCH METHODS**

A comprehensive literature search was carried out using the following keywords: "environment, stress, embryo, chicken" in the databases of PubMed, NCBI, and Google Scholar. All journal papers submitted to the database must be free of financial or other forms of bias, and research must focus on the areas of "environment, stress, embryo" to be eligible for inclusion. Articles are reviewed for data if they meet the inclusion criteria; otherwise, they are eliminated. This also followed PRISMA 2020 rules.

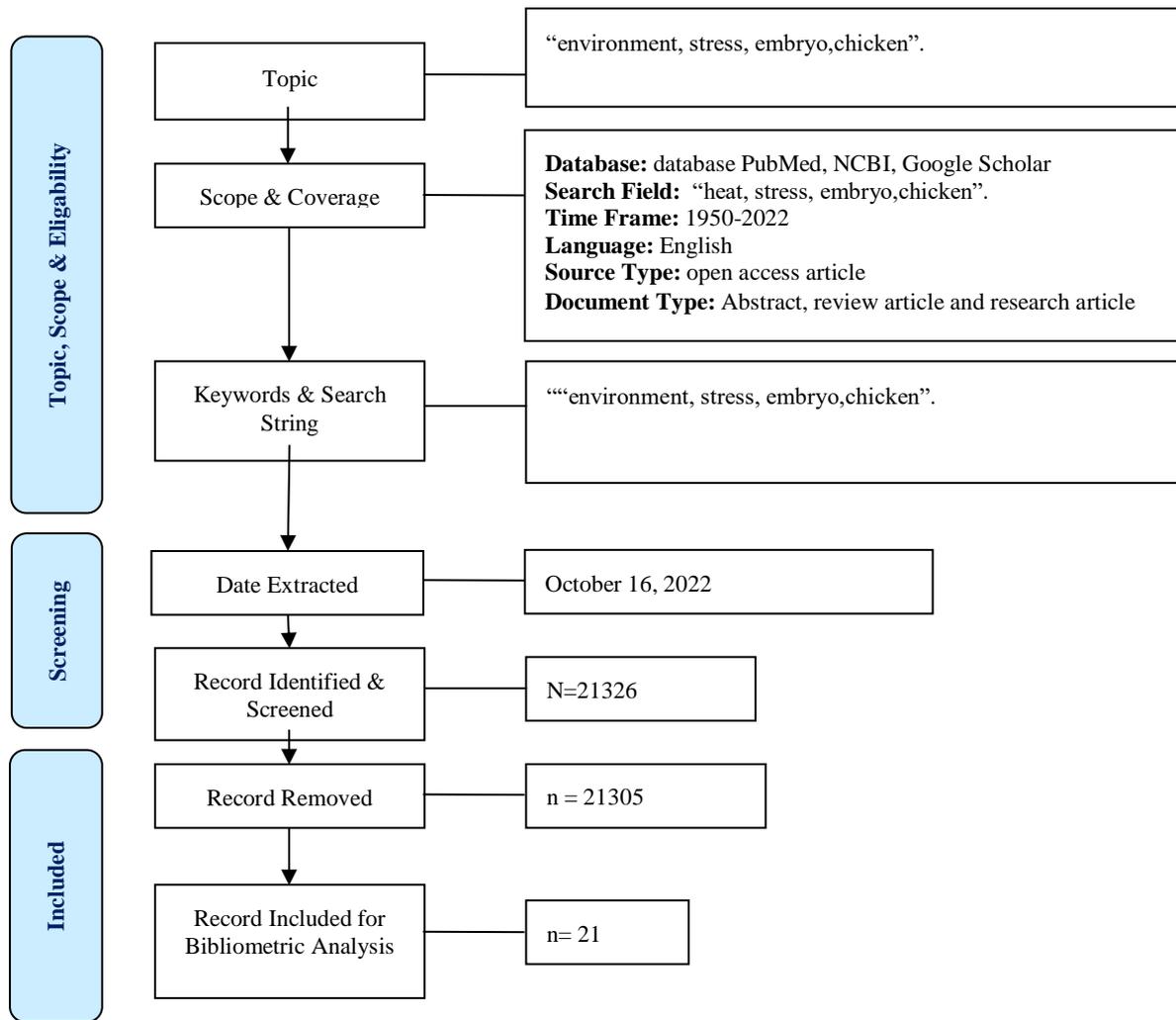


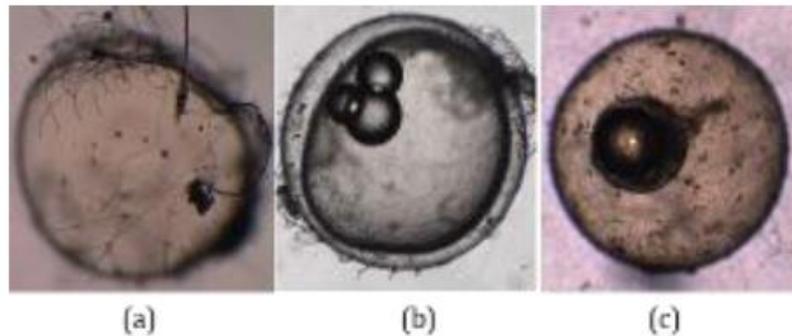
Figure 1. Research strategy flow diagram.

Table 1. Research strategy keyword.

Environment	Stress	Embryo
"ambient"OR"atmosphere" OR"climate"OR"clime"OR "context"OR"contexture" OR"environs"OR"medium" OR"milieu"OR"mise-en- scène,"OR"setting"OR"surr ound"OR"surroundings"OR "terrain	"pressure"OR"strain" OR"t ension"OR"accent"OR"acce ntuation"OR"emphasis"OR "underscoring"OR"weight"	"budding"OR"germinal"OR"inf ant"OR"ancient"OR"early"OR" primal"OR"primeval"OR"primit ive"OR"primordial"OR"aged" OR"age- old"OR"antediluvian"OR"antiqu ated" OR"antique"

#### 4. RESULTS AND DISCUSSION

Using predefined keywords, 21326 articles were retrieved for the terms "environment, stress, embryo, chicken" from the PubMed, NCBI, and Google Scholar databases. After re-selection according to inclusion and exclusion criteria, a total of 21 articles were found to be suitable for inclusion.



**Figure 2** “Exposure to salinity in eggs (a) unfertilized eggs, (b) gastrula at 10-12 ppt treatment, (c) shriveled eggs.

**Table 2.** Embryogenesis time per phase in the control group and the treatment group Salinity.

Stadium	Development time			
	0 ppt	2-4 ppt	4-8 ppt	10-12 ppt
Cleavage	1'20"	1'20"	1'20"	1'20"
Morula	5'10"	5'10"	5'10"	5'10"
Blastula	11'9"	10'38"	10'25"	10'
Gastrula	14'26"	14'28"	14'30"	14'1"
Organogenesis*	1H 7'52"	1H 7'12"	1H 8'36"	1H8'16"
Organogenesis**	4H15"	3H20'38"	4H4'21"	4H10'6"
Hatch	7H19'45"	7H14'20"	7H22'12"	8H1'16"

H: day, ':hour, ":minute, \*:beginning \*\*:end

**Table 3** Effect of zinc sulfate on mortality of broiler chicken embryos (Tegel Tm 70) incubated until day 13.

Age Incubation (days)	ZnSO <sub>4</sub> Dosage per egg (mg)	Number of eggs fertilized	Number of embryos live on day '13	Number of embryos die before day 13 (%)
2	0,0	22	1 8	4 (18,2)
	0,2	23	1 6	7 (30,4)

	0,5	31	1 1	20 (64,5)**
	0,9	33	5	28 (84,8)**
	0,0	25	23	2 ( 8,0)
4	0,2	25	20	5 (20,0)
	0,5	25	20	5 (20,0)
	0,9	25	20	5 (20,0)
	0,0	25	22	3 (12,0)
6	0,2	24	20	4 (16,4)
	0,5	23	1 8	5 (21,4)
	0,9	25	22	3 (12,0)

**Table 4** Effect of zinc sulfate on bone length and femur length of broiler chicken embryos (Tegel Tm 70) that live at 13 days of incubation.

Age Incubation (days)	ZnSO <sub>4</sub> . Dosage per egg (mg)	Number of eggs fertilized	Long (mm) @		index @ long part which resonate (Vy)
			the part that resonate	Bone (y)	
2	0,0	18	6,95 ± 0,98ac	10,32	0,67 ± 0,04a
	0,2	16	7,27 ± 0,234a	±1,084a	0,67 ± 0,03a
	0,5	11	6,44 ± 0	10,85 ± 0,544a	0,67 ± 0,03a
	0,9	5	0,95bc	9,63 ± 1,26b #	0,64 ± 0,02a
				6,01 ± 1,43b	9,39 ± 2,08b #
4	0,0	23	6,22 ± 0,48ab	9,59 ± 0,56a	0,65 ± 0,04a
	0,2	20	5,96 ± 0,55b	9,69 ± 0,76a	0,62 ± 0,333b
	0,5	20	6,43 ± 0,484a	9,96 ± 0,36a	#
	0,9	20	6,20 ± 0,54ab	10,01 ± 0,81a	0,65 ± 0,03a
					10,23 ± 0,69a
6	0,0	22	7,14 ± 0,51a	11,07 ± 0,49a	0,70 ± 0,04a
	0,2	20	7,52 ± 0,464a	##	0,67 ± 0,03a
	0,5	18	7,48 ± 0,49a	10,89 ±	0,69 ± 0,03a
	0,9	22	7,21 ± 0,62a	0,69a#	0,70 ± 0.02a
					10.3 ± 1.19ac

@ : Average  $\pm$  my deviation

a , b , c : significantly different sign of LSR test results for rows in the same column

# : significantly different ( $p < 0.05$ ) compared to the LSR test control

## : significantly different ( $p < 0.01$ ) compared to the LSR test control

**Table 5** Effect of zinc sulfate on the cross-sectional area of the reinforcing section and the area cross-section of femur bone of broiler chicken embryo (Tegel Tm 70) that lived at 13 days of incubation.

Age Incubation (days)	ZnSO <sub>4</sub> . Dosage per egg (mg)	Number of eggs fertilized	Long (mm) @		index @ long part which resonate (Vy)
			the part that resonate	Bone (y)	
2				0,92 $\pm$ 0,20a	
	0,0	18	0,71 $\pm$ 0,16a	0,94 $\pm$ 0,13a	0,79 $\pm$ 0,04a
	0,2	16	0,66 $\pm$ 0,17a	0,89 $\pm$ 0,19a	0,68 $\pm$ 0,03a
	0,5	11	0,64 $\pm$ 0,22a	#	0,70 $\pm$ 0,03a
	0,9	5	0,37 $\pm$ 0,09b	0,69 $\pm$ 0,21b	0,55 $\pm$ 0,02a
4				##	
	0,0	23	0,59 $\pm$ 0,12a	0,79 $\pm$ 0,12a	0,74 $\pm$ 0,04a
	0,2	20	0,58 $\pm$ 0,14a	0,79 $\pm$ 0,14a	0,73 $\pm$ 0,333b
	0,5	20	0,57 $\pm$ 0,10a	0,83 $\pm$ 0,10a	#
	0,9	20	0,56 $\pm$ 0,10a	0,81 $\pm$ 0,09a	0,69 $\pm$ 0,03a
6				1,06 $\pm$ 0,69a	
	0,0	22	0,79 $\pm$ 0,19a	1,03 $\pm$ 0,49a	0,75 $\pm$ 0,04a
	0,2	20	0,71 $\pm$ 0,21a	##	0,68 $\pm$ 0,03a
	0,5	18	0,52 $\pm$ 0,16b##	0,89 $\pm$ 0,69a#	0,58 $\pm$ 0,03a
	0,9	22	0,54 $\pm$ 0,14b##	0,97 $\pm$ 1.19ac	0,55 $\pm$ 0.02a

@ : Average  $\pm$  my deviation

a , b , c : significantly different sign of LSR test results for rows in the same column

# : significantly different ( $p < 0.05$ ) compared to the LSR test control

## : significantly different ( $p < 0.01$ ) compared to the LSR test control

**Table 6.** An overview of research on hens' exposure to cold stress.

CS conditions	TN conditions	Duration	Age	Breed	Findings	Location
15 °C	25 °C	12 h/day for 4 days	17 d old	Ross 308 Broilers	CS predisposes broiler chicks to necrotic enteritis	Greece
2 to 8 °C	25 °C	3–4 h; 8 h (third wk to sixth wk)	3 d, 4 d, 3 wk old	Commercial Broilers	Highest ascites-related mortality	India
16 ± 1 °C	28 ± 1 °C	72 h	10-day-old male	Arbor Acres Broilers	Reduced growth performance; increased blood endotoxin	China
13–15 °C	29–22 °C	28 days	14 d old	Ross 308 Broilers	Decreased beneficial microbes; increased pathogens; oxidative cell damage	Iran
6 ± 2 °C	20 °C	12, 24, 72 h	6 wk old	Chinese indigenous (Huainan Partridge chicken)	Caused oxidative stress	China
12 ± 1 °C	25 °C	Acute CS (1, 3, 6, 12, 24 h); Chronic	15 d old	Broilers	Caused duodenum oxidative stress	China

		CS (5, 10, 20 d)				
-9 to -15 °C	22 °C	24–32 h	5 wk old, 6 wk old	Broilers	Reduction in meat quality	Canada
-17.5 to 27.0 °C	7.4 to 26.5 °C	21 wks	18 wk old	Bashang Long-tail chicken; Rhode Island Red; cross- bred (Layers)	Affected egg production performance	China
12 ± 1 °C	25 °C	Acute CS (1, 3, 6, 12, 24 h); Chronic CS (5, 10, 20 d)	2 wk old	Broilers	Caused intestinal lesions; altered immune function of chicken intestine	China

**Table 7.** a compilation of studies on the stress caused by stocking density on chickens.”.

Stocking density	Age (days)	Duration	Floor area	Breed	Findings
9, 12 (birds/m <sup>2</sup> )	1	42 days	1 m <sup>2</sup>	Male Cornish Cross cockerels	High SD decreased growth performance; increased glutathione concentration in plasma, breast, and thigh of growers
9 (LSD), 18 (HSD) (birds/m <sup>2</sup> )	21	21 days	4.216 m <sup>2</sup>	Ross 308 broilers	Decreasing broiler performance

25, 30, 35, 40 (kg/m <sup>2</sup> )	1	35 days	5.57 m <sup>2</sup>	Ross × Ross 708 male chicks	Increasing SD beyond 30 kg/m <sup>2</sup> adversely affects growth responses and meat yield 30.4 SD decreased BWG; increased intestinal barrier dysfunction; 25.3 SD had no detrimental effects on growth performance
15.2, 20.2, 25.3, 30.4 (birds/m <sup>2</sup> )	1	28 days	0.5928 m <sup>2</sup>	Ross 308 broiler chicken	Poor FCR and lower antibody titer against Newcastle Disease
10 (NSD), 16 (HSD) (birds/m <sup>2</sup> )	–	42 days	0.5 m <sup>2</sup>	Cobb broiler chicks	Tracking 16 m <sup>2</sup> thicker than CSD
8, 10 (SD), 12, 14, 16 (birds/m <sup>2</sup> )	22	38 days	8 m <sup>2</sup>	Lingnan Yellow feathered broilers	Increasing SD decreased the final BW, fat content, and abdominal fat quality
14, 21, 28 (birds/m <sup>2</sup> )	42	49 days	1 m <sup>2</sup>	Maize Yushan chickens	HSD decreased FCR, daily weight gain, GPX in the liver
14 (LSD), 20 (HSD) (birds/m <sup>2</sup> )	21	21 days	1 m <sup>2</sup>	Arbor Acres broilers	No changes to immune parameters; increasing SD decreased final BW
12.5, 17.5, 22.5 (birds/m <sup>2</sup> )	28	13 days	4 m <sup>2</sup>	Suqin yellow chickens	HSD decreased BW; no significant effect on FCR, BWG, and FCR
12.9, 18.6 (birds/m <sup>2</sup> )	21	21 days	1.43 m <sup>2</sup> (12.9); 1.55 m <sup>2</sup> (18.6)	Arbor Acres broilers	HSD affected meat quality
9, 12 (birds/m <sup>2</sup> ); 25, 35	1	49 days	0.11 m <sup>2</sup> (9); 0.08 m <sup>2</sup> (12); 0.935 m <sup>2</sup> (25);	Ross 308 male broilers	

(kg/m <sup>2</sup> ) on			0.715 m <sup>2</sup>		
D49			(35)		
15, 18 (birds/m <sup>2</sup> )	22	29 days	5 m <sup>2</sup>	Arbor Acres broilers	HSD decreased growth and FCR
28 or 40 (kg/m <sup>2</sup> )	1	42 days	0.54 m <sup>2</sup>	Arbor Acres broilers	Decreased final BW, ADG, and ADFI
6, 6.7, 8 (birds/m <sup>2</sup> )	133	16 wks	20 m <sup>2</sup>	Beijing You Chicken (pullet)	8 birds/m <sup>2</sup> adversely affected the performance and welfare of chickens

According to **Figure 2., Table 2., Table 3.; Table 4., Table 5.; Table 6., and Table 7.** during the cell division phase, which lasts for around two to three minutes, the embryo develops rapidly. The blastomere enters the morula phase of cell division when the cell count hits 32 and continues to create an equal number of smaller cells until the cell count reaches 32 again. As the blastomer condenses, a little blastodisc will develop. Newly formed embryonic cells keep growing during the gastrula phase. More defined and palpable body outlines are now visible (Diana et al., 2017; Gusrina, 2014; Redha et al., 2014). After the gastrula phase ends, the process of organogenesis begins, and it is the last stage of embryogenesis. During organogenesis, the most striking changes are the development of pigmentation, the acceleration of the heart rate, the enlargement of the eyes, and the expansion of the body. The egg has an ovary, the head and tail are discernible, and the development of the eye sockets begins, as described by Redha et al. (2014), Annur et al. (2016), and Herjayanto et al. (2017) (Annur. et al., 2016; Herjayanto et al., 2016; Redha et al., 2014).

Salt tolerance affects the embryonic period, as stated by Mostofa (2020) (Mostofa, 2020). Research by Mubarokah et al. (2014) found that because the egg expends a lot of energy just to keep its osmotic pressure constant, the hatching process would be slowed down if the external osmotic pressure differed from what was within the egg (Mubarokah et al., 2014). Hadid et al. (2014) found that embryonic activity, which affects the time it takes for eggs to hatch, is affected by the salt content of the water (Hadid et al., 2014). Heltonika claims that isoosmotic eggs help with embryo development and egg longevity (2014) (Heltonika, 2014).

The most common form of zinc sulfate is in its hydrate form. In the production of rayon, this chemical is mostly used as a thickening agent. Animal feed, fertilizers, and other agricultural applications are just a few of the many that benefit from zinc sulfate supplements. One possible solution to the problem of roof moss is to use zinc sulfate or a comparable zinc compound. Electrolyte for zinc plating; astringent and emetic in medicine; fur and leather preservation; Chvapil & Misiorowski, 1980; Devlin, 1981 (Chvapil & Misiorowski, 1980; Devlin, 1981).

Taking zinc supplements with meals may reduce copper levels in the blood and tissues. Enzyme disruption during metal-metal transit in the intestines and competition amongst metals for the N-S "ligand" are the reasons for this action. In young mice, mineralogy seems to reduce lysyl oxidase activity. Zinc has an effect on ossification in two stages. First, by substituting copper with zinc, the enzyme lysyl oxidase becomes less active, hence inhibiting collagen formation. Both zinc additions boosted collagenase's activity as a zinc metalloenzyme, which in turn improved the resorption of the collagen matrix (Sano & Bana, 1985; Devlin, 1981; Chvapil & Misiorowski, 1980) (Chvapil & Misiorowski, 1980; Devlin, 1981; Iguchi & Sano, 1985). These findings also supported by **Supplementary Table 1**.

## **5. CONCLUSION AND SUGGESTIONS**

Searches in PubMed, NCBI, and Google Scholar using predefined keywords yielded 21 articles out of 21326 that met the inclusion requirements. Environmental stress effects fetal development, according to this study's results. Zinc sulfate and salt affect embryogenesis processes that lead to bone formation. Additional study is necessary to fully understand the impact of various types of stress on embryonic development in various animal species.

## **THANK-YOU NOTE**

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