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Effect of embryonic and post-hatch photo-stimulation with variable light sources on hatchability, endocrine parameters and growth performance in broiler chicken

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ABSTRACT

The objective of this study was to investigate the effects of embryonic and post-hatch photo-stimulation with variable light sources with respect to hatchability parameters, hormonal profile and growth performance of commercial broiler chicken. Uniform sized Cobb broiler eggs (174) were procured from commercial hatchery and incubated in three different groups with arrangement of variable colour light source [Control group; Red light photo-stimulated (675 nm); Green light photo-stimulated group (575 nm) of light]. After hatching, as per earlier grouping, chicks hatched out from respective groups reared under continuous lighting in normal, red, green light up to six week of age in standard management condition in battery cages. The result of the present study indicated that photo-stimulation of incubated eggs with different lights sources had no significant effect on hatchability percentage and hatching time. Green light photo-stimulated group showed significantly higher body weight gain with better feed conversion ratio than red and control groups from 0 to 6 wk of age. Feed intake did not differ significantly within the groups. Green light photo-stimulation promotes growth performance traits via stimulating circulating level of gonadal axis and somatotrophic axis hormone. The results of the study provide evidence that green light photo-stimulation used in this study is beneficial in terms of improved growth performance without affecting hatchability in broiler chicken.

Keywords: Broiler chicken, Embryonic photo-stimulation, Gonadal axis hormone, Growth performance, Hatchability, Somatotropic axis hormones

Due to significant development in genetic selection of broiler chicken, present broiler birds achieve optimum body weight (1.5–2.0 kg) in relatively short growth periods (35–42 days). So period of incubation (21 days) becomes nearly 50% of the productive life span of commercial broiler chicken. Incubation period is becoming important window period for application of any scientific technique for successful rearing of broiler birds. So during incubation stage, it is essential to maintain incubator environment at optimum level with respect to temperature, humidity, ventilation and egg turning for getting healthy chicks. In commercial hatchery, usually eggs are incubated in dark condition so that the hatching eggs are exposed to light only during opening and closing of door for routine hatchery operation. Fairchild and Christensen (2000) suggested light as a possible fifth environmental variable, which is not monitored during the incubation of avian eggs. During incubation period from 3rd day of embryonic age, avian embryo can respond to light (Erwin *et al.* 1971). Lighting hours and timing of light exposure can significantly affect the embryo's physiological traits, hatchability, chick quality and post-hatch performance (Ozkan *et al.* 2012, Archer and Mench 2013).

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During post-hatch period, various external factors such as housing condition of broiler birds should be at optimum to get expected benefits. Usually broiler birds are reared in litter or in cages in half walled poultry sheds. Lighting is one of the most important environmental factors which directly influence the production of reared birds. Previous studies of researchers, established that the light (duration, intensity and wavelength) is possibly the major environmental stimulus affecting physiology, behaviour, immunity and growth rate of birds. Earlier studies on the impact of variation in light intensity and wavelength reported profound effects on growth performance of broilers (Rozenboim *et al*. 1999, Lewis and Morris 2000, Rozenboim *et al.* 2004). In poultry housing, variation in light during the brooding period can result in poor performance and low profitability. In broilers, beneficial effect of green light exposure on early age growth is mediated by enhancing the proliferation of skeletal muscle satellite cells and the expression of a growth hormone receptor gene (Halevy *et al*. 2006).

Varied results on hatchability parameters and growth performance has been reported earlier depending upon different type of artificial illumination system used (source, colour, intensity, wavelength, period, frequency of light), species, age, strain of broiler bird used in experiments (Cao

et al. 2008). Although growth performance benefits reported earlier in studies, there is limited information regarding physiological mechanism by which lighting exerts its favourable effect on growth performance of broiler. Further, most of the studies have evaluated the effect of photo-stimulation with variable light in different phases of broiler production either in pre-hatch period (incubation period) or post hatch rearing. So it is necessary to test the efficacy of photo-stimulation with different lighting systems for post hatch performance in broiler chicken in complete life cycle of broiler chicken, i.e. embryonic and post hatch period. In the current study, the growth performance and physiological development of commercial broiler chicks was investigated, in response to photo-stimulation with different lights during embryonic and post hatch period. Therefore, in view of the aforementioned, this experiment is designed to study the effect embryonic and post hatch photo-stimulation with variable light sources on hatching parameters, growth performance and hormonal profile in broiler chicken.

MATERIALS AND METHODS

This experiment trial work was carried out at Experimental Livestock Unit of the institute. The animal experimental procedure was carried out as per the guidelines and approval of Institute Animal Ethical Committee (IAEC) of the Institute.

Experimental design

Incubation and photo-stimulation: Uniform sized cobb broiler eggs (174) were procured from commercial hatchery and incubated with the dry bulb temperature ranging from 37.22–37.77°C and wet bulb temperature of 29.44–30.55°C from day 1 to 18 in three different groups with arrangement of variable colour light source [Control group; Red light photo-stimulated group red (675 nm); green light photo-stimulated group (575 nm) of light]. On day 18, all the unfertile eggs were removed after candling and the fertile eggs were shifted to hatching trays. The relative humidity was increased by setting the wet bulb thermometer reading of more than 32.22°C till hatch. Hatchability percentage, hatching time and chick weight at hatch were recorded.

Birds and housing: After hatching, as per earlier grouping, hatched chicks from respective groups reared in red, green light up to six week of age in standard mangemnetal condition in battery cages. A total of 105 hatched chicks, i.e. 35 chicks for each group were taken and divided into five replicates (5 replicates with 7 chicks in each replicate). The feed and fresh drinking water were provided *ad lib.* during the entire experimental period. Experimental diets were prepared with maize and soybean meal as major ingredients (Table 1).

Growth performance: Growth performance were recorded biweekly wise in terms of body weight (BW), body weight gain (BWG), feed intake (FI), feed conversion ratio (FCR) and livability of the birds. The experimental diets were given *ad lib.* and the residue was weighed at biweekly interval in order to arrive at feed intake. Based on the data pertaining to the feed intake and body weight gain, cumulative feed conversion ratio (FCR) was calculated. Daily monitoring and recording on individually basis had been carried.

Table 1. Ingredient and nutrient composition of experimental diets

	Pre-starter (0–7 days)	Starter (8–21 days)	Finisher (22–42 days)
Ingredients (%)			
Maize	56.81	58.53	62.41
Soybean meal	37.00	36.10	32.00
Fat/ oil	2.20	1.80	2.10
Lime stone	1.00	1.00	1.10
Di calcium phosphate	1.75	1.75	1.50
Salt	0.35	0.35	0.35
Lysine	0.40	0.10	0.12
Methionine	0.24	0.12	0.15
Vit. Min. premix*	0.25	0.25	0.27
Nutrient composition (%)			
ME (kcal/kg)	2,996.00	2,994.00	3,051.00
CP	22.51	21.97	21.27
Lysine	1.52	1.27	1.15
Methionine	0.55	0.51	0.46
Threonine	0.81	0.68	0.66
Calcium	0.99	0.99	0.97
Phosphorus, avail.	0.45	0.45	0.40

*Trace mineral premix 0.1%, Vit. Premix 0.1%, B-Complex 0.02%, Choline 0.05%. Trace mineral premix supplied mg/kg diet: Mn, 75; Se, 0.2; Fe, 40; Zn, 70; Cu, 10. The vitamin premix supplied per kg diet: Vit. A, 8,250 IU; Vit. D_3, 1,200 ICU; Vit. K, 1 mg; Vit. E, 40 IU; Vit. B_1, 2 mg; Vit. B_2, 4 mg; Vit. B_{12}, 10 mcg; niacin, 60 mg; pantothenic acid, 10 mg.

Analysis of hormones: Blood samples were collected by superficial venepuncture of the brachial vein starting from 2nd week onwards at weekly intervals and continued until the end of experimental period at 6th weeks of age. Samples were analyzed for testosterone and estradiol-17β with radioimmunoassay kits obtained from Immunotech, France. The intra and inter coefficient variation for testosterone and Estradiol-17β were 4.45% and 8.12% respectively with sensitivity of the hormone 0.01 ng/ml per tube. Methodology was followed as per the manufacturer procedure provided in the kits.

Chicken growth hormones (*cGH*) and Insulin like growth factor-I (*IGF-I*) hormones were estimated by enzyme-linked immunosorbent assay (ELISA) as per the manufacturer's protocol. The cross reactivity of *cGH* antisera against *IGF-I* was less than 0.01%. The intra and inter assay coefficients of variation were less than 9%. The sensitivity of the assay was 0.002 ng/ml. The cross reactivity of the *IGF-I* antisera against *cGH* was more than 0.01%. The intra and inter coefficient variation for *cGH* and *IGF-I* were less than 11%.

Statistical analysis: The observations were analyzed using the Statistical Package for Social Sciences (SPSS, 2010 Version 18.0). Different parameters with respect to hatchability, hormone assay and growth performance traits

between control and treatment groups was determined by one way analysis of variance (ANOVA) for completely randomized design. Significance between the treatments tested by employing Tukey's HSD Post-hoc test. The means of different groups were considered significant at P≤0.05.

RESULTS AND DISCUSSION

Importance of light management during incubation is became one of the basic requirement for getting good and healthy quality of chicks. Stress level, corticosterone levels, bilateral physical asymmetry of hatched chicks significantly reduced under lighted incubation in compare to dark incubation (Archer and Mench 2013). It is always beneficial to get post-hatch chicks with minimum stress level which can withstand better during transportation stress from hatchery to farm.

Hatchability parameters: Photo-stimulation of incubated eggs with different lights sources had no significant effect on hatchability percentage on fertile egg basis and hatching time within the treatment groups (Table 2). Use of incandescent bulbs as light source during incubation may depress the hatchability and increase embryo mortality during incubation due to extra heat production in incubator (Rozenboim *et al.* 2003). Deviation of eggshell temperature from the optimum results in change of embryonic temperature, reduced hatchability, organ development and chick growth (Michels *et al.* 1974, Decuypere 1979, Taylor 2000, Meijerhof 2003, Shafey 2004). In this study, non significant effect on hatchability percentage with different photo-illumination which indicated that used light sources did not produce excess heat in incubator.

These findings on hatchability are consistent with the findings of earlier researchers (Rozenboim *et al.* 2004, Archer *et al.* 2009, Zhang *et al.* 2016). Rozenboim *et al.* (2004) observed monochromatic green light exposure of broiler hatching eggs did not affect hatching time or hatchability percentage in compare to dark incubated group. Exposure of cobb broiler eggs to different lighting treatment continuous light (24L:0D); no light (0L:24D); 12 h of light (12L:12D) during incubation did not influence hatching time, hatching percentage (Archer *et al.* 2009). Hatchability percentage, hatching time, chick embryo weight did not differed significantly due to exposure of green or white LED light in during incubation in Arbor Acres broiler birds (Zhang *et al.* 2016). Hatchability percentage did not differed significantly due to use of different lighting regimen with LED lights (12 L; 12 D) and complete darkness during incubation although LED light exposed group had better chick quality than the Dark group (Huth and Archer 2015). Physical dimensions of the eggs and pigmentation of eggs, type and amount of light reaching the embryo are important factors for obtaining favourable response in terms of hatchability parameters and embryonic development of incubated eggs under photo-illmunation with different light sources (Shafey 2004, Veterany *et al.* 2007, Huth and Archer 2015). However, these findings were not in agreement with earlier studies with different type of light sources. Exposure of white light during incubation had better hatchability in comparison to red, blue monochromatic light exposed broiler eggs (Veterany *et al*. 2007, Hluchy *et al.* 2012). lighted incubation with white LED light (18 or 21 days) in comparison to dark incubated eggs, lighted incubated group showed improved hatchability, higher numbers of chick with healed navels and chick with no abnormalities in comparison to dark incubated group (Archer 2015). Exposing hatching eggs with combination of white and red LED light (12 Light:12 Dark hour) during incubation improved hatchability of broiler birds by 5 percent and lowered post-hatch fear response compared to birds hatched in the dark or provided green LED light (Archer 2017).

Table 2. Hatchability and chick weight

Group	Egg wt (g)	Chick wt (g)	Hatchability (%)
CL-PGrp	63.03	42.86	87.50 (42/48)
RL-PGrp	62.96	43.21	85.71 (42/49)
GL-PGrp	62.52	46.37	87.23 (41/47)
SEM	0.78	0.686	0.056
Significance	0.776	0.01	

(CL-PGrp, Control group; RL-PGrp, Red light photo-stimulated group; GL-PGrp, Green light photo-stimulated group).

Chick weight at hatch: Embryonic photo-stimulation of incubated broiler eggs with green light significantly influenced chick weight at hatch. GL_PGrp groups had significantly higher body weight (46.37 gm) in comparison to RL_PGrp (43.21 gm) and C_Grp (42.86 gm) at hatch (Table 2). Earlier studies reported favourable effect of photo-stimulation with green lights during incubation in broiler chicken (Shafey *et al*. 2002, Rozenboim *et al*. 2004). Pre and post-hatch growth performance was significantly improved in terms of body weight and pectoral muscle percentage in green light incubated broiler breeder in comparison to dark incubated eggs, also observed that green light incubated embryos grow faster in terms of daily weight gain from 5th day of incubation (Shafey *et al.* 2002). Body weight and pectoral muscle percentage improved significantly in the embryos and post-hatch birds incubated under monochromatic green light in comparison to dark incubation (Rozenboim *et al.* 2004). Commercial broiler hatching eggs incubated under red light had higher body weight of hatched chicks (47.64 gm average body weight, compared to white LED light hatched chicks 46.29 gm) with lower number of leg defects (4% less) in compare to dark control group chicks (Archer 2015).

Growth performance: Growth performance parameters, i.e. biweekly body weight, body weight gain, feed intake, feed conversion ratio of photo-stimulated birds with variable light groups are presented in Table 3.

Photo-stimulation of GL_PGrp chicks group showed significantly (P<0.05) higher body weight gain (2,508 gm) than RL_PGrp (2,423) and C_Grp (2,437) groups during entire study period, i.e. 0–6 wk. Feed intake did not differ significantly within the groups in entire study period of 0 to 6 wk. Green light photo-stimulation significantly

Table 3. Effect of photo-stimulation with different lights on growth performance in broiler chicken

Treatment	CL-PGrp	RL-PGrp	GL-PGrp	Mean±SE
Biweekly body weight (*g*)				
0 wk	42.86±0.81^{b}	43.21±0.21^{b}	46.38±0.05^{a}	44.15±0.36
2 wk	432.75±0.53^{b}	432.91±3.11^{b}	440.81±2.09^{a}	435.49±1.91
4 wk	1322.27±4.84^{a}	1319.71±11.47^{a}	1316.92±10.85^{a}	1318.32±9.01
6 wk	2480.4±30.21^{ab}	2466±20.18^{b}	2554.22±19.01^{a}	2500.21±23.13
Body weight gain (*g*)				
0–2 wk	389.90±1.30	389.69±3.32	394.43±2.05	391.34±2.22
2–4 wk	889.52±5.34	886.81±10.09	876.11±1.10	884.15±5.51
4–6 wk	1158.12±28.06^{b}	1146.91±9.15^{a}	1237.3±23.63^{a}	1180.78±20.28
0–6 wk	2437.54±29.77^{ab}	2423.41±20.32^{b}	2507.85±19.02^{a}	2456.27±23.04
Feed intake (*g*)				
0–2 wk	562.2±1.26^{b}	553.97±1.62^{a}	555.67±1.37^{a}	557.28±1.42
2–4 wk	1441.75±7.43	1455.12±4.67	1446.05±4.88	1447.64±5.66
4–6 wk	2460.67±3.80^{b}	2442.4±2.59^{a}	2443.62±3.39^{a}	2448.9±3.26
0–6 wk	4464.62±11.63	4451.5±5.72	4445.35±6.23	4453.82±7.86
Feed conversion ratio				
0–2 wk	1.44±0.05	1.42±0.016	1.41±0.01	1.42±0.03
2–4 wk	1.62±0.01	1.64±0.01	1.65±0.02	1.64±0.01
4–6 wk	2.13±0.05^{b}	2.13±0.03^{b}	1.98±0.03^{a}	2.07±0.04
0–6 wk	1.83±0.012^{b}	1.83±0.01^{a}	1.77±0.01^{a}	1.81±0.01

CL-PGrp, Control group; RL-PGrp, Red light photo-stimulated group; GL-PGrp, Green light photo-stimulated group). Values represent means±standard error; ^{a,b,c} Means bearing different superscripts in a row differ significantly (P<0.05).

influenced feed conversion ration within the treatment groups. GL_PGrp birds showed better FCR (1.77) in comparison to RL_PGrp (1.83) and C_Grp birds (1.83) from 0 to 6 wk of age.

In concurrence to our obtained findings on post-hatch growth performance, most of the earlier works have reported embryonic photo-stimulation with green light improved growth performances in broiler chicken (Halevi *et al.* 1998, Rozenboim *et al.* 2003, 2004., Cao *et al.* 2008). In earlier studies, involving embryonic exposure of green light to hatching eggs during incubation, increased proliferation and differentiation of adult myoblasts and altered the pattern of myofiber formation which resulted in higher skeletal muscle development during incubation and the posthatch period (Halevi *et al.* 1998). Favourable effect was reported by embryonic green light photo-stimulation of turkey embryo during incubation in terms of increased weight gain and muscle growth in comparison to control group (Rozenboim *et al.* 2003). Rozenboim *et al*. (2004) observed embryonic photo-stimulation of hatching eggs in broiler chicken, increased muscle development rate especially pectoralis muscle as percentage of body weight and gained more body weight than control birds reared in dark condition during 0–6 week of age in broiler birds. Further they concluded that higher weight gain in green embryonic photo-stimulated birds due to enhancement of proliferation and differentiation of embryonic myoblasts and subsequent muscle hypertrophy. Cao *et al.* (2008) in their studies on Arbor Acres broilers reared under white, red, green and blue lights from light-emitting diode lamps as light sources, green light reared birds had higher body weight with better FCR during the early period of growth (0 to 26 d of age). At later stages from 38th day up to 49th day blue light reared broilers had larger body weight than other groups with lowest FCR (2.36). In contrary to above results, Archer (2015) observed non significant effect on weight gain and feed conversion ratio at 45 day of age in broiler chicken on exposure of white LED light during incubation (18 or 21 days) in comparison to dark incubation.

Variable result obtained in with different studies light exposure probably due to the use of different light source, light schedule, light intensity, strain of broiler birds, pigment concentration of hatching eggs, hatching environment of incubator (Shafey *et al.* 2005, Cao *et al.* 2008, Archer 2015)

Gonadal axis Hormone: Results of plasma Testosterone and Estradiol-17β level of embryonic photo-stimulated with variable light groups are presented in Table 4. In this study, embryonic photo-stimulation with green light significantly affected plasma testosterone and Estradiol-17β level of post-hatch birds than RL_PGrp and C_Grp during entire study period. In line with obtained findings in present study on plasma testosterone level, exposure of green light enhanced secretion of testosterone and myofiber growth that led to increased growth and productive performance during 0 to 26 days of age in broiler chicken, however later period (27–49 day) blue light exerted similar stimulatory effect (Cao *et al.* 2008). Earlier studies also explained effect of testosterone level on muscle accretion and maintenance of muscle mass and force (Crowley *et al.* 1996, Sinha-Hikim *et al.* 2003, Axell *et al.* 2006). Estradiol-17β combined with testosterone increased osteoblast proliferation and alkaline phosphatase activity, accelerated the osteoblast cell cycle of chicken which is important for normal bone cell development and tissue formation. (Chen *et al.* 2010).

Table 4. Effect of photo-stimulation with different lights on growth performance in broiler chicken

Treatment	CL-PGrp	RL-PGrp	GL-PGrp	Mean±SE
Growth hormone level (*pg/ml*)				
14th day	4.12±1.01[b]	3.91±0.95[c]	4.92±1.05[a]	4.31±1.00
21th day	4.16±0.86[c]	4.86±0.92[b]	6.75±0.28[a]	5.25±0.69
28th day	4.34±0.35[c]	5.09±1.03[b]	8.13±0.44[a]	5.85±0.61
35th day	5.21±1.15[c]	6.01±0.64[b]	9.11±1.11[a]	6.77±0.97
42th day	5.71±0.97[c]	6.11±0.44[b]	9.16±0.85[a]	6.99±0.75
IGF-I concentration (pg/ml)				
14th day	10.19±1.11[c]	11.94±1.23[b]	14.94±0.32[a]	12.35±0.89
21th day	11.96±1.03[c]	12.47±1.19[b]	18.22±1.09[a]	14.21±1.10
28th day	12.41±0.96[c]	12.02±1.08[b]	20.95±1.21[a]	15.12±1.08
35th day	12.75±1.21[c]	14.12±0.98[b]	20.15±1.14[a]	15.67±1.11
42th day	12.51±0.99[c]	14.61±1.10[b]	22.94±1.36[a]	16.68±1.15
Testosterone concentration (*pg/ml*)				
14th day	71.12±1.15[c]	92.91±0.95[b]	118.92±1.05[a]	94.31±1.05
21th day	72.86±1.94[c]	101.76±0.92[b]	174.55±0.28[a]	116.39±1.05
28th day	72.91±1.39[c]	105.99±1.03[b]	172.13±0.44[a]	117.01±0.95
35th day	75.52±1.30[c]	112.66±0.64[b]	189.63±1.11[a]	125.93±1.01
42th day	80.97±1.99[c]	116.92±1.74[b]	142.69±1.85[a]	113.52±1.86
Estradiol-17β concentration (*pg/ml*)				
14th day	20.66±1.24[b]	19.69±1.01[c]	59.29±0.53[a]	33.21±0.93
21th day	23.29±1.29[b]	22.11±1.10[c]	67.93±1.19[a]	37.77±1.19
28th day	26.39±0.91[c]	30.02±1.19[b]	72.22±1.32[a]	42.87±1.14
35th day	32.14±0.16[c]	34.12±0.69[b]	76.31±1.09[a]	47.52±0.65
42th day	32.61±0.99[c]	41.69±1.12[b]	76.66±1.16[a]	50.32±1.09

CL-PGrp, Control group; RL-PGrp, Red light photo-stimulated group; GL-PGrp, Green light photo-stimulated group). Values represent means±standard error; [a,b,c]Means bearing different superscripts in a row differ significantly (P<0.05).

Somatotropic axis hormones: In this study, embryonic photo-stimulation with green light significantly elevated *cGH* and *IGF-I* level of post-hatch birds than RL_PGrp and C_Grp during entire study period Table 4.

For normal growth and development of chick embryo, optimum concentration of circulating growth hormone is essential. Exposure of broiler eggs with green light during incubation significantly influenced *cGH* and *IGF-I* levels during early post-hatch period, optimum levels of *cGH* and *IGF-I* are important factors that increase the early post-hatch performance and muscle mass of birds (Zhang *et al.* 2014). Earlier studies also reported that lighting stimuli enhanced *cGH* secretion from pituitary somatotroph cells of male broilers and compensatory growth in broilers was associated with an amplification of *cGH* secretary burst mass (Kuhn *et al.* 1996).

IGF-I is an important regulating factor in cellular proliferation and differentiation, satellite cell proliferation, DNA synthesis and tissue growth (Florini *et al.* 1996). Chicks reared under monochromatic green light had higher level of circulating *IGF-I* levels with rise in chick satellite cell myogenic processes during early post-hatch stages in comparison to white or red light reared chicks (Liu *et al.* 2010). *IGF-I* stimulates the proliferation and DNA synthesis in chicken muscle satellite cells (Florini *et al.* 1996), enhances glucose and amino acid uptake and protein synthesis and inhibits protein degradation in chicken muscle cells (Duclos *et al.* 1999) subsequently induces the hypertrophy of adult skeletal muscle (Adams and McCue, 1998, Chakravarthy *et al.* 2000). Photoperiodic lighting during incubation is favourable for better adaptation, reduction of adaptive stress by inducing melatonin rhythms and altering secretion hypothalamic-pituitary-adrenal axis hormone than their dark-incubated counterparts during incubation period (Ozkan *et al*. 2012).

In conclusion, our results indicated that green light embryonic photo-stimulation improved body weight of hatched chicks without affecting hatchability. In addition embryonic and post-hatch photo-stimulation with green light improved body weight gain with better feed conversion ratio. Green light photo-stimulation promotes growth performance traits via stimulating circulating level of circulating gonadal axis and somatotrophic axis hormone. The results of the study provide evidence that green light photo-stimulation used in this study is beneficial in terms of improved growth performance without affecting hatchability in broiler chicken. Future studies are warranted to understand effect of green light photo-stimulation on metabolic pathways involved in embryonic development during embryogenesis.

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