EFFECT OF IN OVO INJECTION OF NANO IODINE ON EMBRYONIC DEVELOPMENT, VIABILITY, THYROID ACTIVITY, AND HATCHABILITY IN BROILER CHICKENS

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SUMMARY

odine is one of the trace minerals besides manganese, selenium, copper and iron that are important nutrients required at small amounts for normal growth and development of the chicken embryo. In the current trail the effects of inovo injection of nano iodine (Nano-I) during early incubation on embryonic mortality, hatchability, chick quality and some anatomical parameters in broiler chickens were investigated. A total of 500 hatching eggs of Ross 308 broiler breeders were numbered and weighed (average 65±0.5g.) then incubated at 99.8°F and 55% R.H. On the 7th day of incubation, eggs were candled, and infertile eggs were excluded. The fertile eggs were distributed into 4 treated groups (100 eggs/treatment) as follows; C: un-injected eggs (control); sham: amnion sac was penetrated by a needle only, and two injected groups with 0.05 and 0.1 ml of 0.9 % nano-I (T1 and T2, respectively). Results showed a highly significant increase in the total embryonic mortality during both the incubation and hatching periods, especially during the mid-stage of incubation (8 to18 days) in Nano-I-injected groups compared with control groups. Incubation period duration increased significantly in injected a group that was dose-dependent (487.2 and 504 hours in T1 and T2, compared with 480 and 472.2 hr, in sham and control groups, respectively). Tona score was significantly higher in control 94% compared with 92% in sham and 84% in nano-I injected eggs. While chick weight, chick length and yolk free body mass % (YFBM %) had no significant differences between all treatments. Some anatomical observations were noted among the treatments. Duodenum, pancreas, thymus, spleen, and bursa of Fabricius were more developed in chicks that hatched from 0.05 ml nano-I group compared with those injected by 0.1 ml nano-I or control groups and this is very evident in the thyroid glands. There is no significant difference between treated groups in plasma level of T4 while nano-I injected groups were higher in plasma level of T3. It is concluded that; in ovo injection with nano-I at doses of 0.05 and 0.1 ml had a negative effect on hatchability and embryonic viability without significant effects on chick quality except for Tona score. More studies are needed to determine the optimal dose of nano-I for in ovo injection in broiler chickens.

Keywords: In ovo injection, nano iodine, embryonic mortality, hatchability, chick quality, broiler chickens.

INTRODUCTION

The requirements of nutrients, especially the trace minerals for broiler breeders are recommended according to strain and age of the breeder. Deficiencies of specific trace minerals can be induced in developing avian embryos by adding insufficient amounts of the minerals to laying hens ration (Richards and Steele, 1987a). Trace minerals deficiency can cause bad growth, abnormal organogenesis, and in extreme deficiencies, death of the embryo occurs (Savage, 1968; Richards and Steele, 1987a). Conversely, excessive amounts of trace minerals can be detrimental to the developing embryo. Avian embryos develop in ovo without access to maternal nutrient supply. So, all the nutrients, including trace minerals, required for the normal embryo development are deposited by the hen into the egg during its formation (White, 1991). On the other hand, many factors are affecting the deficiency

of nutrients in egg components due to reduction in feed intake that caused by diseases and stressors. Deficiency in trace minerals in egg components caused a negative effect on normal embryonic development. So, the in ovo injection during embryonic development is very crucial. Assuming that in ovo nutrition with trace minerals during embryonic development will be recovering the deficiency and improving the metabolic processes during the incubation.

Differences in the results of in ovo injection applications are dependent on many factors such as type of the injected material, dose of injection and the embryo age. A few studies were conducted on the in ovo injection of nano particles of the trace minerals. El-Deep *et al.* (2020) conclude that immune response, antioxidant activities and hatchability % were improved with in ovo injection of nano selenium in amnion sac at 18 days of incubation. Similar results on broiler chicken injected in amnion sac at 17 days of incubation with nano selenium and nano zinc were recorded to cause an increase in GSH-Px, SOD, total protein and a decrease in both glucocorticoids and thyroid hormone levels (Meisam *et al.*, 2019). Nano selenium injected in air cell of quail eggs, significantly improved hatchability % but embryonic mortality was increased (Dhyaa *et al.*, 2020).

Embryonic growth and development were improved with nano-iron in ovo- injected at 7 days of incubation in air cell (Amal, 2018). Myogenesis of broiler chicken embryo was enhanced with 20 ppm of nano silver injection into air cell (Walaaet *et al.*, 2021). Iodine is integral to the formation of the thyroid hormones that affect cellular metabolism. Iodine deficiency in breeder diets cannot maintain the hormonal balance (Rashmi *et al.*, 2014). Injection of excess potassium iodine in albumin before incubation increased the thyroid weight at the 18th day and inhibited the thyroid follicles development (Guo *et al.*, 1991). There are no studies assessing the effects of in ovo injection of nano iodine on the embryonic development, and some hatchability traits in broiler chickens.

Therefore, the aim of the present study was to evaluate the embryonic development, hatchability and chick quality as a consequence of in-ovo nano iodine injection of broiler breeder eggs.

MATERIALS AND METHODS

A total of 500 eggs were collected from Ross 308 broiler breeders aged 46 weeks. The eggs were stored for 3 days in refrigerator at 18 °C and 85% R.H., and then they were placed at room temperature (25 °C and 50 R.H.) for 3 hours.

Individual egg weight was recorded using electronic balance and average egg weight was estimated. Eggs that weighed 65±0.5 grams were incubated in automatic incubator at 99.8 °F and 55% R.H. Throughout the incubation period, all eggs were incubated according to the common routine procedures. For the last three days of incubation (hatching period) eggs were subjected to 99.0 °F and 80 % % R.H.

Experimental design and protocol within this study were conducted according to ethical guidelines approved by the Experimental Animal Care and Research Ethics Committee of Ain Shams University, Agriculture Sector Committee (Approval No 5-2023-6).

Egg injection:

On the 7th day of incubation, all eggs were candled to obtain the fertile eggs. Infertile eggs were excluded. Total of 400 fertile eggs were divided into four experimental groups as follows: C: un-injected eggs (control); sham: eggs were punctuated in amnion sac without injection any material and T1 and T2: eggs were injected with 0.05 and 0.1 ml of 0.9 % nano-I respectively.

Injection procedure:

As shown in Fig. (1): Air cell for each egg was detected by candling and marked by pencil, then the embryo position was determined depending on the site of embryo eyes, then the position was circled by pencil. By using an electric driller, air cell was pierced at about 2-3 mm from the edge of air cell over the embryo position. A Syringe of 1 ml volume supported with 24 G x1 $\frac{1}{4}$ needle was used in injection to ensure that it was reached to the amnion sac (Fig. 2).

As shown in Fig. (3): before injection of nano iodine, Indian ink was used for injection to ensure that the needle was penetrated the amnion sac. Before injection eggs were cleaned with 70% ethanol and sealed immediately after injection using non-toxic glue then sticky paper ($\frac{1}{2} \times \frac{1}{2}$ cm) were put on the glue to ensure complete close of the hole then injected eggs were returned to the incubator. In parallel, the non-injected group was taken out of the incubator and kept in the same environmental conditions of injected eggs treatments.

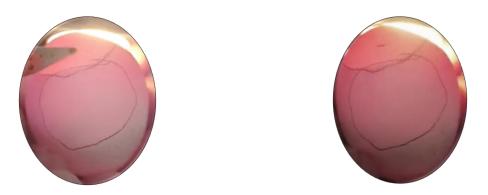


Fig. (1): Air cell was detected under candling and marked by pencil, then the embryo position was determined depended on the site of embryo eyes and the position was circled by pencil





Fig. (2): Air cell was pierced at about 2:3 mm from the edge of air cell over the embryo position (c).





Fig. (3): Indian ink was injected to ensure that needle was penetrated the amnion sac.

On the 18th day of incubation all eggs were candled to obtain the embryonic mortality percentage and the number of eggs that contained dead embryos were recorded, and then excluded. Time of hatch was estimated as number of hours from the start of incubation till the end of the full hatching in all treatments. After hatching, breakout analysis for unhatched eggs was done. A total of 10 chicks from each treatment were taken, the Tona score was recorded, and blood samples were collected using 3ml 3 syringes by heart puncture and then eviscerated to examine the internal organs and thyroid glands.

At hatch, about 2 cm from the duodenum from 5 chicks per treatment were carefully dissected, rinsed with saline (0.9% NaCl), and fixed in a 12% formalin solution. Thin transverse sections (4-5 micron) were cut using standard paraffin embedding procedures and mounted on glass slides, stained with the ordinary hematoxylin and eosin stain (H & E) according to Bancroft and Stevens (1990). The histological technique was performed at the Pathology Laboratory, National Cancer Institute, Cairo University, Egypt. Histological sections were examined by using a routine light microscope (OPTIKA, Model B-193) provided with a digital microscope camera (OPTIKA, Model C-B) under magnification powers of x10 and x40.Blood

Type of nano-iodine:

Xodine (Zodine) is a mono element base as nano- colloid iodine 0.9 %, USA, was bought online.

Time of injection:

The optimal time for Nano iodine injection was determined according to Wentworth and Ringer (1986) they reported that thyroid glands start to originate at 5 days of embryonic development from the floor of the pharynx. Also, iodinated tyrosine molecules have been demonstrated within the developing chick thyroid gland at approximately the same time as the initial appearance of follicles (ED 9-11) of incubation (Daugeras *et al.*, 1976). Wentworth and Ringer (1986) reported that the thyroid of the chicken embryo at 7 days of incubation can concentrate the radioactive iodine many times greater than the amount present in the blood. Also, the thyroid gland is believed to function after the 10th DOI and fall under hypothalamic-pituitary regulation around the 11th day (Thommes, 1987)

Site of injection:

Amnion sac was the main injection site of minerals at an early stage compared with air sac according to Peebles, (2018). Also, in ovo injection of nano particles of minerals was performed amnion sac as the main target of injection (El-Deep *et al.*, 2020; Meisam *et al.*, 2019).

Statistical analysis:

Statistical analysis was carried out using General Linear Model (GLM) procedures by SAS (2010) using simple one-way analysis of variance. Significant differences among treatment groups were tested using Duncan's multiple range tests, Duncan, (1955). Statistical significance was determined at p < 0.05.

RESULTS AND DISCUSSION

The data of the current study showed that a significantly higher mortality rate was recorded for embryos in injected groups compared with controls (negative and sham control). Total embryonic mortality was increased in each of 0.05 or 0.1 ml nano-I dose injection (57.5 and 70.3 % in T1 and T2 resp.) compared with 11 and 19 % in control and sham groups respectively (Table 1).

Table (1): Effect of in ovo nano Iodine injection on embryonic mortality rate and hatchability % in broiler chickens:

	Т	reatments			Sign.
Parameters	Control	Sham	T1 (0.05 ml nano I)	T2 (0.1 ml nano I)	
Embryonic mortality%				,	
Early stage (0-7 days)	4.4% ^b ±0.25	4.0 ^b ±0.24	12.5% ^a ±1.55	14% ^a ±2.01	**
Mid stage (8-18 days)	2.2% ^b ± 1.25	6.2 ^b ±1.5	33.75% ^a ±2.36	46.9% a±3.21	**
Late stage (19: 21 days)	4.4%1.02 °±	9.1 ^b ±1.6	11.25% ^a ±1.45	9.4% ^b ±0.35	**
Total embryonic mortality	11% ^b ±1.5	19.3 ^b ±2.5	57.5% ^a ±5.4	70.3% ^a ±6.25	**
Hatchability %					
From no. fertile eggs	79.8% ^a ±3.21	69.2 ^a ±2.0	40.8% b±2.31	20% °±1.02	**
From no. total eggs	75% ^a ±2.31	64.1 ^a ±1.0	36% ^b ±3.01	17% °±2.22	**

a,b,c: Means within a column with different superscripts are significantly different (P < 0.05). Sig. = Significance, * (P < 0.05), NS = not significant.

Most of the embryonic mortality rate occurred during the mid-stage period of incubation (8-18 DOI) where the injected groups recorded 33.75 and 46.9 % for T1 and T2 compared with 2.2 and 4 % in control and sham groups respectively (Table 1). Dead embryos during this stage were examined to determine the time of mortality and its relation to the time of injection. Data based on the optical examination showed that most percentage of the mortality occurred between 14-18 DOI (about 7-11 days after injection) (Fig. 6). Also, the results of egg candling after injection for 3 consecutive days showed highly viability of chicks from in ovo injected treated groups. Increasing mortality rate especially at mid-stage may be caused by the dose on nano-I injection. These results agree with Dhyaa et al., (2020) reported that nano selenium injected in air cell of quail eggs significantly improved hatchability % but embryonic mortality was increased. (Savage, 1968; Richards and Steele, 1987a). Also,

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excessive amounts of trace minerals can be detrimental to the developing embryo. No data is available for the recommended dose of nano-I during embryonic growth stage. Many researchers studied the effects of in ovo injection of nano particles of some trace minerals such as zinc, selenium, iron, copper and silver in broiler chickens (El-Deep *et al.*, 2020; Meisam *et al.*, 2019; Dhyaa *et al.*, 2020; Amal 2018; Walaaet *et al.*, 2021 and Guo *et al.*, 1991), an improvement of embryonic development was reported in all studies.

In the chick embryo, the yolk is the main supply of iodine that necessary for normal thyroid function, stored in the form of iodide (Daugeras-Bernard *et al.*, 1993). During incubation, iodide is transferred across the yolk sac and gradually increased as development proceeds, with plasma iodine levels remaining low until ED-9 and then increasing until hatching (Daugeras-Bernard and Lachiver, 1980). Data illustrated in Table 1 showed a highly significant decrease in hatchability (%) for nano-I injected groups compared with control and sham groups. The hatchability (%) is dependent on the embryonic viability which decreased with nano-I injection. In this study both

hatchability and viability were nano-I dose-dependent manner, where the dose of 0.05 ml nano-I had slightly negative effects on hatchability and viability than 0.1 ml nano-I.

Table (2): Effect of in Ovo nano Iodine injection on incubation period duration (hours), Tona score, Chicl	ĸ
weight (g), Chick length (cm) and YFBM %.	

					Sig.
Parameters	Treatments				_
	Control	Sham	T 1 (0.05 ml	T 2 (0.1 ml	_
			nano I)	nano I)	
Length of incubation p. (hrs)	475.2 ° ±0.25	480 ^a ±2.32	487.2 ^b ± 3.25	504 °± 4.28	**
Tona score.	94% ^a ±1.02	90 ^a ±2.25	$84\% \ ^{b} \pm 4.25$	84% ^b ±2.14	**
Chick weight (g)	42.58 ± 2.03	42 ± 3.02	42.6 ± 5.21	42.5 ± 4.21	NS
Chick length (cm)	19.4 ± 1.08	19 ± 2.05	19.1 ± 3.25	19.2 ± 2.14	NS
YFBM %	89.75 ± 2.05	88±2.35	88.6 ± 6.24	$89.4{\pm}5.47$	NS
1 36 111 1 110	· ·		(D 0.05) CI	a	D 0.01

a,b: Means within a column with different superscripts are significantly different (P < 0.05). Sig. = Significance, * (P < 0.01), NS = not significant.

Breakout analysis for un-hatched eggs showed that dead embryos at late-stage (18-21 DOI) in injected nano -I groups were fully developed without signs in the sham group that reflect that the injection procedure had no effects on embryonic mortality (Fig. 4 A). Signs of trauma or accident were noted in nano-I injected groups at the dose of 0.1 ml (Fig. 4 B).

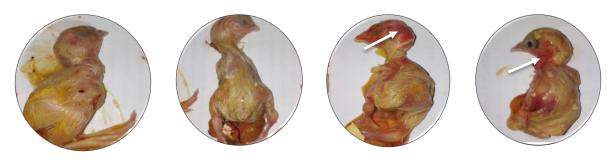
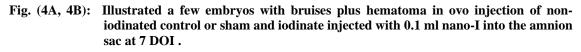


Fig. (4A)

Fig. (4B)



Length of the incubation period (hrs.) was increased in 0.1 ml injected groups (504) followed by 0.05 injected groups (487.2) compared with 475.2 and 480 in sham and control groups, respectively (table 2). On the other hand, tona score was decreased by about 10 degrees compared with the control with no significance of naon-I injected on chick weight, chick length and yolk free body mass (YFBM %).

The samples of evicerated hatching chicks showed some anatomical observations between treatments. Duedenum, pancreas and gall blader were larger and well develped in 0.05 ml and 0.1 ml nano-I groups compared with both controls (Fig. 5). Furthermore, similar observation was noted for the primary lymphoid organs (bursa of Fabricius and thymus) and spleen. Also, the thyroid glands are larger in two nano-I groups than controls. These data agree with Daugeras-Bernard *et al.*, (1993) who stated that iodine injected at excess amount (500 μ g) in yolk sac at the 2 th DOI induced an increase in thyroid weight to two folds than control and showed thyroid goiters with a much-flattened epithelium surrounding enlarged colloid-filled vesicles. Also, a decrease was observed in embryo weight in in-ovo iodinated groups than controls.

These results agree with the findings of El-Deep *et al.* (2020) who noted that immune response improved with in-ovo injection of nano selenium with an increase in GSH-Px, SOD, total protein and decrease in glucocorticoids and thyroid hormone levels (Meisam *et al.*, 2019).

Our data indicated that the effect of in-ovo injection is probably more dependent on nano-I dose and time of inovo injection, besides, iodine metabolism during embryo development. Daugeras-Bernard *et al.* (1993) reported that allantoic fluid contains iodine levels in excess higher than in plasma and the balance between iodine levels in these two embryonic fluids is maintained throughout development. Also, iodine excretion via the chorioallantoic membrane into allantoic fluid is an important mechanism for regulating plasma iodine concentrations.

Data of plasma components of one day old chicks are presented in Table 3. Significant increase was determined in triglycrides and cholesterol levels in inoculated groups, but the total protein concentration was decreased compared to sham and control groups.

Table (3): Effect of in ovo injection at 7 days of incubation with Nano iodine in broiler chicken eggs on
plasma constituents and thyroid hormones level at hatch.

Treatments					Sign.
Traits	Control	Sham	T1 (0.05 ml	T2 (0.1 ml	
			nano I)	nano I)	
Total protein (g/dl)	2.60 ^a ±0.39	2.55 ^a ±0.19	1.92 ^{ab} ±0.039	1.73 ^b ±0.039	*
Uric acid (g/dl)	$6.60^{a} \pm 1.52$	65 ^a ±1.12	6.84 ^a ±0.23	4.56 ^b ±0.42	*
ALT (u/L)	130.95 ^a ±4.39	128.95 ^a ±4.2	134.73 ^a ±12.16	116.66 ^b ±2.82	*
AST (u/L)	228.24±9.04	224.24±3.1	291.58±3.49	257.24±65.17	NS
Cholesterol (mg/dl)	249.55±9.16	244.22 ± 4.11	259.24±11.90	258.39±12.55	*
Triglycerides (mg/dl)	238.88 ^b ±	235.55 ^b ±	244.44 ^{ab} ±28.04	$250.00^{a} \pm 10.18$	*
	38.3	23.2			
Plasma T3 ng/ml	$20.36^{b} \pm 0.43$	20.12 ^b ±0.15	21.81 ^{ab} ±0.68	23.66 ^a ±1.09	*
Plasma T4 ng/ml	3.35±0.06	3.12±0.06	3.10±0.17	3.39±0.25	NS
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a,b: Means within a column with different superscripts are significantly different (P < 0.05). Sig. = Significance, * (P < 0.05), NS = not significant.

There are no significant differences in plasma T4 level concentration, while inoculated groups had a higher value for T3 than sham and control groups.

Histological examination of the duodenum is illustrated in Fig (6, 7, and 8). Results showed that an increase in the villi height and the number of goblet cells also were increased in inoculated groups compared with sham and control groups.

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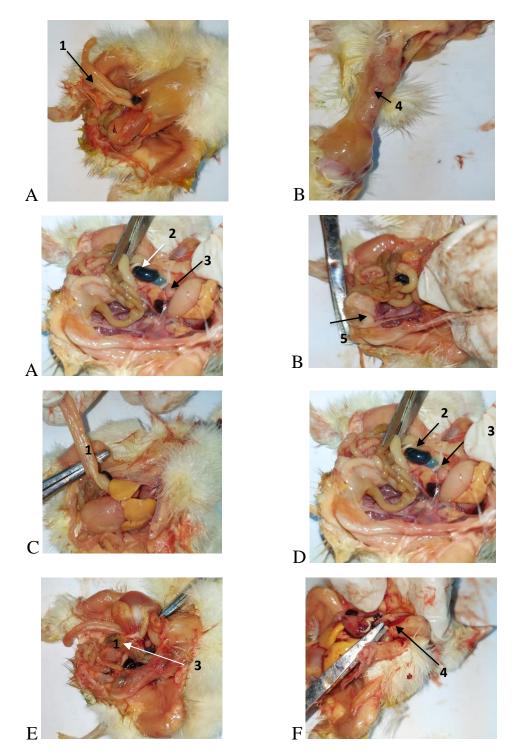


Fig. (5): Illustrated Duodenum, pancreas (1), gall blader (2), spleen (3), thymus (4) and bursa (5) were larger and more developed in 0.05 ml (a and b) and 0.1 ml nano-I (c and d) group compared with controls (e and f).

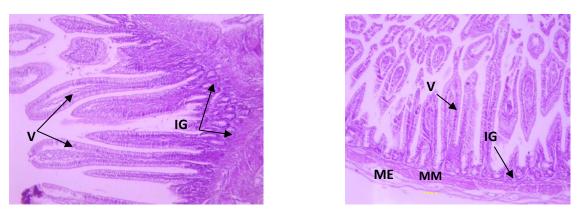


Fig. (6): Histology of Duodenum of the control group at 18 ED . Here, Villi (V), Intestinal gland (IG), Muscularis mucosae (MM), Muscularis externa (ME) and Serosa (S) are shown.. H & E ×400 (A) and x 100 (B)

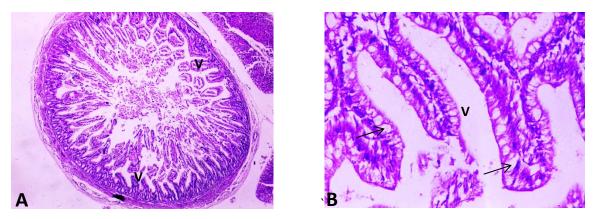


Fig. (7): Histology of the duodenum of the in ovo injection with 50 μl nano iodine at 18 ED. Here, Villi (V), Intestinal gland (IG), Muscularis mucosae (MM), Muscularis externa (ME), goblet cells (black arrows) and Serosa (S) are shown. H & E ×100 (A) and x 400 (B)

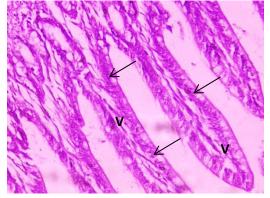


Fig. (8): Histology of the duodenum of the in ovo injection with 0.1 ml nano iodine at 18 ED. Here, Villi (V), goblet cells (black arrows) are shown. H & E ×400.

CONCLUSION

In-ovo injection with 0.9 % nano-I at doses of 0.05 and 0.1 ml had a negative effect on hatchability and embryonic viability with no significant effects on chick quality except Tona score. More studies are needed to determine the optimal dose of nano-I for Ovo injection of broiler eggs.

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تأثير حقن البيض بجزيئات اليود متناهية الصغر علي التطور الجنيني والحيوية ونشاط الغدة الدرقية نسبة الفقس في دجاج إنتاج اللحم.

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كلية الزراعة الصحراوية والبيئية، جامعة مطروح، مطروح، 51512، مصر.

يعتبر اليود أحد العناصر المعدنية الصغري إلى جانب المنغنيز والسيلينيوم والنحاس والحديد التي تعتبر مهمة ومطلوبة بكميات صغيرة النمو الطبيعي وتطور جنين الدجاج. تم دراسة تأثير حقن بيض الدجاج بجزيئات اليود متناهية الصغر (I-Nano) أثناء التطور الجنيني علي النفوق الجنيني ، نسبة الفقس ، وجودة الكتاكيت وبعض الصفات التشريحية في دجاج انتاج اللحم . عدد 500 بيضة من أمهات دجاج انتاج اللحم سلالة روص 308 متوسط وزن (65±0.5 جم) تم تحضينها عند 99.8 درجة فهرنهايت و 55 رطوبة نسبية. عند اليوم 7 من التحضين ، تم عمل فحص ضوئي للبيض وتم استبعاد البيض الغير مخصب. تم توزيع البيض المخصب على 4 مجموعات معالجة (100 بيضة / معاملة) على النحو التالي ؟ كنترول: بيض غير محقون (كنترول سالب) ؟ Sham : ما ختراق كيس كيس الأمنيون بواسطة إبرة الحقن ومجموعتين محقونتين بـ 0.50 و 0.1 مل من 0.9 ما ما معان (100 م

أظهرت النتائج زيادة معنوية للغاية في إجمالي النفوق الجنيني خلال التطور الجنيني، خاصة خلال المرحلة المتوسطة من التغريخ (8 إلى 18 يوما) في المجموعات المحقونة بالنانو مقارنة بالمجموعات الكنترول والشام. زادت فترة التفريخ بشكل ملحوظ في المجموعات المحقونة مرتبطة بالكمية المحقونة (47.2 و 504 ساعة في 71 و 72 ، مقارنة ب 480 و 472.2 ساعة في المجموعات المحقون ، على التوالي). كان مقياس Tona score أعلى بكثير في الكنترول 94٪ مقارنة ب 92٪ في Sham و 82% في المجموعات المحقون ، على التوالي). كان وطول الكنكوت وكتلة الجسم الخالية من صفار البيض (47BM٪) لم يكن لها فروق معنوية بين جميع المعاملات. لوحظ بعض الفروق التشريحية بين المعاملات. الاثني عشر والبنكرياس والغدة الثيموسية والطحال وغدة البرسا كانت أكثر تطورا في الكتاكيت التي فقست م دانو يود مقارنة بتلك التي تم حقنها بواسطة 1.0 مل نانو-1 أو مجموعات الكنترول و هذه البيض المحقون بالنانو.

خلصت الدراسة إلي ان حقن بيض الدجاج بجزيئات اليود متناهية الصغر (Nano-I) أثناء التطور الجنيني بجرعات 0.05 و 0.1 مل كان لها تأثير سلبي على نسبة الفقس النفوق الجنيني دون تأثيرات كبيرة على جودة الكتاكيت باستثناء مقياس Tona score . كما اتضح ايضا ان هرمون T4 لم يتغي معنوياً بين جيمع المعاملات علي الكس كان تركيز بلازما الدم من T3 عالي معنوياً في المجموعتين المحقونتين بجزيئات اليود متناهية الصغر . اوضح الفحص الهستولوجي للامعاء زيادة في ارتفاع الخملات و عدد الخلايا الكأسية في المجموعتين المحقونةين بجزيئات اليود متناهية الكترول و Sham . هناك حاجة إلى مزيد من الدر اسات لتحديد الجرعة المثلى لحقن بيض الدجاج بجزيئات اليود متناهية (Nano-I) أثناء التطور الجنيني.