

HIGH DOSE PROGESTERONE EFFECTS THE GROWTH OF EARLY CHICK EMBRYO

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ABSTRACT

Objective: To find out the effect of high dose progesterone on the development of early chick embryo.

Study Design: Lab based randomized controlled trial.

Place and Duration of Study: This study was carried out in Army Medical College and Post Graduate Institute of Poultry Sciences, Rawalpindi from June 2010 –December 2010.

Material and methods: Forty five specific pathogen free, fertile, eggs of Fyoumi species of chick were selected at zero hour of incubation. They were incubated at 37.5 °C and 75% relative humidity for 26 hrs until the embryos reached stage 8 of the development. Then on stage 8 the eggs were divided into three groups consisting of 15 eggs per group. The first group (G1) was incubated without any operation. The second (G2) and third groups (G3) were injected with two and twenty times more than physiologic doses of progesterone respectively. After 48 hours of incubation, all embryos were examined for their development under light microscopy.

Results: All the embryos of G1 and G2 showed normal development according to their stage of development, while 4 out of 11 embryos of G3 were under developed and their survival rate was also less.

Conclusion: Exogenous progesterone at levels twenty times above its physiologic range effects the development of chick embryos. Further studies are needed to explain the mechanisms of this effect.

Keywords: Chick embryo, Development, High dose progesterone,

INTRODUCTION

As the progesterone plays such a important role in the pregnancy, it is known as the "hormone of pregnancy", from low levels allowing unfertilized eggs to slough away with the endometrium in menstruation to the high levels preparing the uterus for the embryo. The mother's immune system is also suppressed by progesterone so that her body will not reject a fertilized egg, it also inhibits uterine contractions to keep the embryo in place, thickens the mucus of the cervical plug to help protect against infection and inhibits the mother from producing milk when she is pregnant¹.

It is the adequate progesterone that prevents the shedding of the uterine lining during the pregnancy. During in vitro fertilization (IVF) if its level is not enough, at the time when embryos are

placed back, can lead to failure of IVF cycle. Since the early days of IVF, progesterone supplementation has been used to make up for the deficit created by removal of these cells². The problem of questionable progesterone availability was further aggravated by the use of the Gonadotropin-releasing hormone (GnRH) agonist in the treatment regimen of women undergoing IVF cycle. GnRH agonists work by preventing premature surges of endogenous LH during an IVF cycle through pituitary suppression, allowing time for a larger number of oocytes to reach maturity prior to harvesting. The use of GnRH agonist causes the suppression of pituitary LH secretion for as long as 10 days after the last dose of agonist. In the absence of this LH signal, the corpus luteum may be dysfunctional, and subsequent secretion of the progesterone and estrogen may be abnormal. In the absence of proper stimulation by progesterone or estrogen, endometrial receptivity may be compromised leading to decreased implantation and decreased pregnancy rates³. In this context, progesterone is usually prescribed during the period of organogenesis, starting at the latter part of the

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Received: 16 Sep 2013; Accepted: 04 Dec 2013

menstrual cycle and continuing till 8-10 weeks of the pregnancy.

Studies on the teratogenic effects of progesterone have dealt mostly with the possible masculinising effect on the female fetus. Other abnormalities that have been described with the use of progesterone are: cardiac malformations, the effects on the central nervous system, like neural tube development defects and development of amelia⁴. The rationale of this study was to find out the effect of high dose progesterone on the development of early chick embryo.

MATERIAL AND METHODS

This lab based randomized controlled trial was carried out at Army Medical College and Post Graduate Institute of Poultry Sciences, Rawalpindi from June 2010 -December 2010.

Forty five fertile, specific pathogen free eggs of Fyoomi species of chick were selected through consecutive non probability sampling at zero hour of incubation. The eggs were collected from post graduate institute of poultry sciences, Rawalpindi. Eggs of abnormal shape, color, size, and texture, or stored in the refrigerator were excluded from the study. The eggs were placed in the hatchery, with the rounded side to the top where the chorioallantoic membrane is situated and rotation of the eggs was done at regular intervals. Day of placement of eggs in hatchery was taken as day zero.

The eggs were incubated at 37.5°C and 75% relative humidity for 26 hrs until the embryos reached stage 8 of development according to Hamburger and Hamilton⁵. At this stage, the eggs were divided at random into three groups consisting of 15 eggs per group. All the eggs were washed with 70% alcohol and properly labeled on the outer shell. The grouping was done as:

G1, [Control Group A] with uninjected eggs.

G2, Eggs injected twice the physiological dose of progesterone.

G3, [Experimental Group] eggs injected with high dose progesterone.

The dosage for the experiment was determined in accordance with procedures described by Pamir et al⁷. Twice the equivalent dose of this level of progesterone was calculated to be 15.7 ng and high dose progesterone, 157 ng, (Water Soluble progesterone, Sigma - Aldrich Comp. Code: P7556, St. Louis, Missouri, USA) was diluted in 0.1 ml of normal saline for each embryo of G2 and G3.

Under laminar flow a hole was made on the outer shell, at the blunt pole of eggs with an egg driller. Using a sterile 28-gauge needle and a tuberculin syringe, 0.1 ml of working solution was injected from the blunt end over the embryonic disc. The hole was sealed with paraffin. Then the eggs were placed back in the hatchery for another 22 hrs.

Embryo collection and the procession was performed in accordance with procedures described by standard textbooks on the subject^{8,9}. After about 48 hours of incubation, the eggs were cracked at air space and the outer shells were chipped out to create a large opening to see the embryo. The viability of the embryos was assessed by the heart beat. All the embryos were fixed with Carnoy's fluid, stained with HCl-carmine¹⁰. They were examined under 20X and 40X with 220560 Olympus, stereomicroscope to assess any gross developmental abnormalities.

Data Analysis

Data was entered on SPSS version 16. Frequency percentages were calculated for the categorical data like alive embryo, dead embryo, fully developed embryo, underdeveloped embryo, embryos failing to develop, fully developed embryos with open neural tube, and normally developed embryos. Chi square test was applied.

RESULTS

Gross examination of embryos under dissecting microscope

Examination under stereomicroscope showed that 7 embryos out of 45 (15.6%) were

failed to develop while 38 (84.4%) embryos were alive. Out of the 7 dead embryos, 1 (6.66%) embryo was from G1, 2 (13.33%) embryos were from G2, and 4 (26.66%) embryos were from G3. Regarding 38 live embryos, 14 (100%) embryos of the G1, 13 (100%) embryos of the G2 and, 7 (63.6%) embryos of the G3, passed characteristics of the stage 12 development, while 4 (36.3%) embryos from G3 were not well developed after 48 hours of incubation. Only live embryos were included in the study.

Chi square test was applied between the total number of dead and alive embryos in control groups, G1 and G2 and experimental group G3 after 48 hours of incubation (p value was not significant 0.22). Chi square test was applied between the total number and frequency of alive but under developed embryos in control groups, G1 and G2 and experimental group G3 after 48 hours of incubation (p value was significant 0.045).

Description of fully developed live embryos

Under dissecting microscope, development of all the embryos of G1, G2, and 63.3% embryos of G3 were according to their stage 12 of development. At that time their 16 somites were seen (Figure-1(a)). The cephalic region of the embryo was twisted in such a manner that the left side comes to lie next to the yolk.

Both neuropores were closed (Figure-1(a)), primary optic vesicles were well established, auditory pits were deep but wide opened (Figure-1(b)) and hearts were slightly S-shaped and was differentiated into 4 compartments, the sinus venosus, connected with the veins, the atrium, the U-shaped ventricle and the bulbus cordis (Figure-1(c)). From the anterior end of heart two ventral aortae were seen. They ran backwards as dorsal aortae.

Description of underdeveloped live embryos

Thirty six percent (36.3%) embryos from G3 were not well developed. Instead of 16 somites, 10 to 12 somites were seen (Figure-2(c)), three primary brain-vesicles were clearly seen (Figure-

2(b)), The sinus rhomboidalis (diamond-shaped) was still present as the only opening of the neural

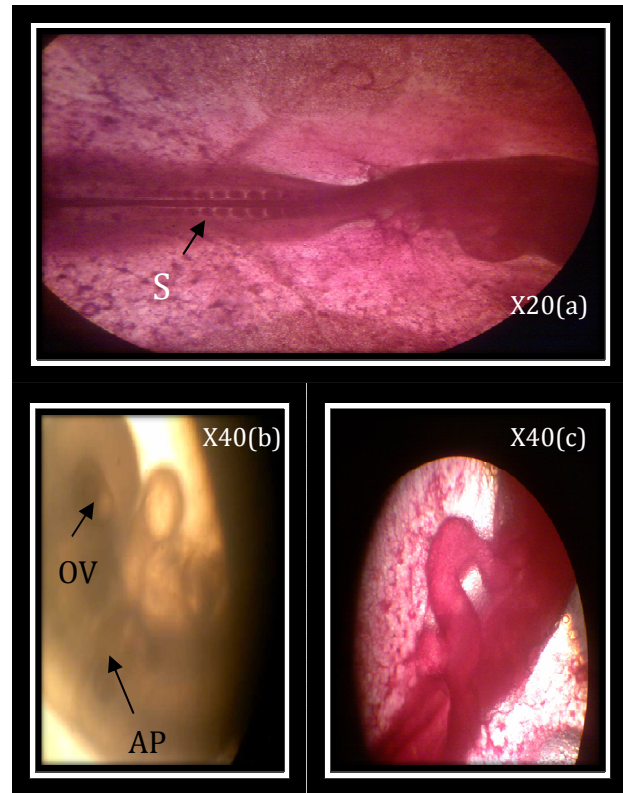


Figure-1: Photomicrographs showing 48 hours fully developed embryos of controlled G2, 16 somites (S) are seen, anterior and posterior both neuropores are closed (1a), primary optic vesicles (OV) are well established, auditory pits (AP) are deep (1b), heart (H) is slightly S-shaped (1c).

tube (Figure-3) and the primitive streak was only rudimentary. The eye vesicles were rather large and hearts were bent slightly to the right (Figure-2(b)).

In the experimental group G3 there was defect in neural tube closure of 6 embryos, 4 (66.7%) embryos were underdeveloped and there was defect in neural tube closure. (Figure-2(a)), while 2 (33.3%) embryos were fully developed but there was a defect in the closure of the neural tube (Figure-3).

DISCUSSION

The major finding of this study is that progesterone in high doses; affect the development of early chick embryos.

In human embryos, the use of natural progesterone is practiced frequently in threatened abortion and the clinical contexts in which natural exogenous progesterone is administered during the period of organogenesis of the embryo are IVF and ICSI. The use is primarily intended to supplement the endogenous production of progesterone by the corpus luteum, and later the placenta, during pregnancy¹¹⁻¹³.

Studies have shown that IVF singletons and twins have significantly increased risks of preterm births, low birth weight and other adverse perinatal outcomes compared to spontaneously conceived singletons^{14,15}. Pregnancies achieved after standard in-vitro fertilization (IVF) bear an increased risk for prematurity and low birth weight¹⁶. In another study done by Jackson et al shows that IVF singleton pregnancies were associated with significantly higher rate of perinatal mortality, preterm delivery, low birth weight, and small for gestational age¹⁷. Thus the findings of the present study are particularly relevant to IVF, which is being performed with increasing popularity all around the world.

Different studies show the effect of progesterone on different stages of development. A study on 18-day-old chick embryos were done which shows, progesterone caused significant reductions in both weight and length and shank weight and length¹⁸. Progesterone negatively affected day 8 embryo development rates in BOEC (Bovine Oviduct Epithelial Cell) system. This negative effect was abolished when the progesterone antagonist was added to the culture medium¹⁹.

Although a large number of studies have failed to show progesterone as a human teratogen²⁰⁻²². The present study found a remarkably higher frequency of death and restricted development in groups of chick embryos exposed to high dose progesterone. Similar findings have been noted in animal models²³. It is compelling to hypothesize that

high dose progesterone may be a factor in

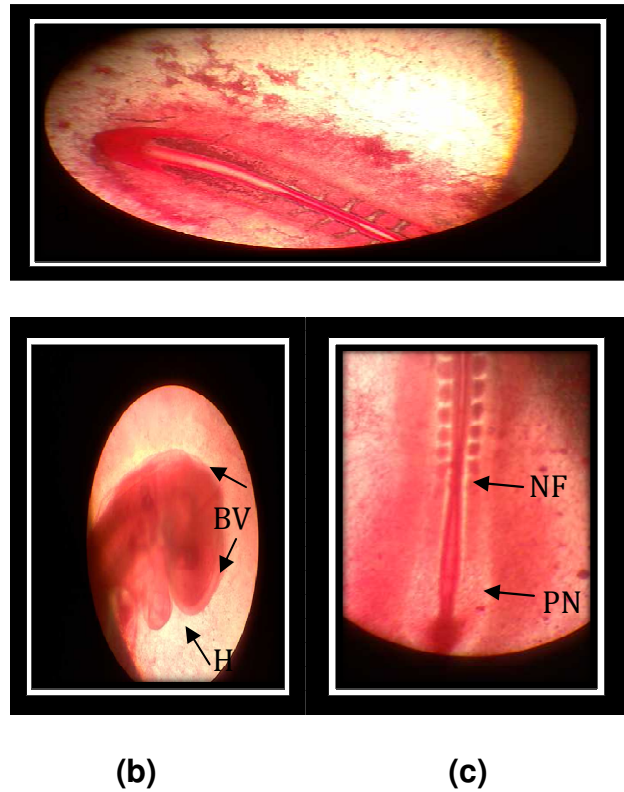


Figure-2: Photomicrograph showing underdeveloped embryos of G3 after 48hrs of incubation, instead of 16 somites (S) 10 to 12 somites are seen (2c), neural folds (NF) failed to meet in the midline, posterior neuropores (PN) are opened (2a), primary optic vesicles (OV) are poorly established, auditory pits (AP) are shallow three primary brain-vesicles (BV) are clearly seen, heart (H) is bent slightly to right (2b). Approx. X40.

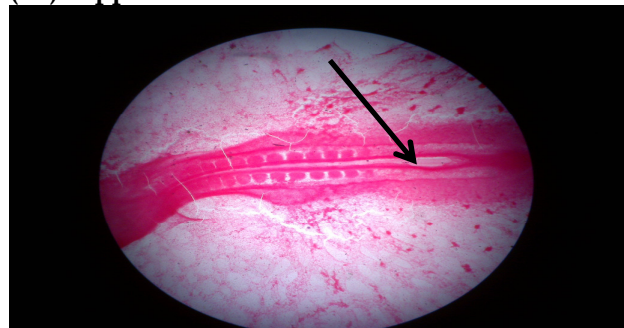


Figure-3: Fully developed embryo of G3 with failure of closure of neural tube. (Arrow= sinus rhomboidalis)

premature birth and early pregnancy loss in

situations where it is administered in supra-physiological doses in humans.

Table-1: Total number of dead and alive embryos in control groups, G1 and G2 and experimental group G3 after 48 hours of incubation.

Groups	Alive embryos	Dead embryos
G1	14 (93.33%)	1 (6.66%)
G2	13 (86.7%)	2 (13.33%)
G3	11 (73.33%)	4 (26.66%)

p value= 0.2262

Table-2: Total number and frequency of alive but under developed embryos in control groups, G1 and G2 and experimental group G3 after 48 hours of incubation.

Groups	Fully developed	Under developed
G1	14 (100%)	0 (0%)
G2	13 (100%)	0 (0%)
G3	7 (64%)	4 (36%)

p value = 0.045 significant.

Table-3: Effect of high dose progesterone on embryos of G3 (group after 48 hrs of incubation).

Group G3 embryos after 48 hrs of incubation	no
Embryos failed to developed	4
Under developed embryo with neural tube defect	4
Fully developed embryo with open neural tube	2
Normally developed embryos	5

CONCLUSION

Exogenous progesterone at levels twenty times above its physiologic range effects the development of early chick embryo.

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