

Understanding in ovo vaccination

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The initial in ovo (in the egg) vaccination research was conducted by United States Department of Agriculture scientists in the early 1980s. In ovo administration of Marek's disease vaccines HVT, SB-1, CV1988 to the late stage chicken embryo has been shown to be safe and to induce earlier immunity than post-hatch administration. The practice of in ovo vaccination was moved from the laboratory to commercial hatcheries in 1992.

Commercial in ovo vaccination began in the United States and today is routine in Southeast Asia, Japan, Australia, Europe, and the Americas. Vaccines for Marek's disease, infectious bursal disease, and fowl pox are routinely administered in commercial broiler hatcheries using the Inovoject system from Embrex Inc.

The complexity of the in ovo route is often under appreciated and this is attributable in part to the five embryonic compartments that can be accessed by the needle during the in ovo vaccination process air cell, allantoic sac (waste fluid), amniotic fluid, embryo body and yolk sac.

Furthermore, embryo body injections can be subcutaneous, intramuscular or intra-abdominal. The efficacy of a vaccine can be affected by the embryonic compartment in which it is deposited.

This is best demonstrated by Marek's disease vaccines, where it has been shown that vaccine delivered to the embryo body or amniotic fluid results in excellent protective immunity, but administration of Marek's vaccine to the air cell or allantoic sac provides poor protection.

It is important to understand the details of commercial and laboratory scale in ovo application of vaccines.

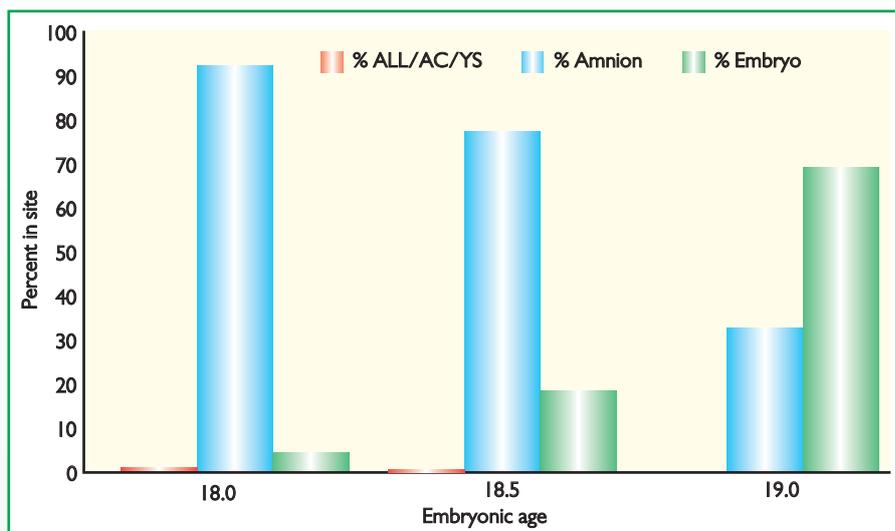


Fig. 1. Percent of embryos injected in various sites at 18.0, 18.5 and 19.0 days of incubation using the Inovoject system. Embryo incubation age was a significant ($P < 0.05$) factor in percent of injection. ALL/AC/YS = allantoic sac, air cell, and yolk sac injections combined.

Studies were conducted to determine the frequency of sites injected and factors that affect in ovo site of injection (route). In a portion of these studies, the Inovoject system was used to deliver dye to broiler embryos.

In other studies, dye was manually delivered using a syringe and needle to broiler and leghorn embryos.

Results from these studies indicate that embryo developmental stage (embryo maturity), embryo incubation age, chicken breed, pre-set egg storage time, needle type, and incubation conditions can influence the in ovo site of injection.

Materials and methods

- **Incubation:** Embryos directly compared for site of injection were incubated simultaneously in the same Jamesway 252 incubator, unless noted. Embryos were candled to remove infertile,

contaminated, and cracked eggs prior to site of injection analyses.

- **Commercial in ovo site of injection.** An in ovo injection machine (Inovoject system) was operated as recommended by the manufacturer.

Embryos were injected with dye at different incubation ages and examined for dye location as described below. A total of 3,983 embryos from Cobb/Cobb, Ross/Hubbard and Ross/Arbor Acres breeders ranging from 32-66 weeks of age were examined.

- **Laboratory (manual) in ovo site of injection.** Specific pathogen free (SPF) leghorn eggs (Charles River SPAFAS) and commercial broiler eggs were prepared for manual injection by punching a hole at the apex of the blunt end of the egg.

A syringe with needle was held vertically and inserted through the punch hole to the hub to deliver 100ul of dye. Comparisons among and within breeds at different incubation times (18.0, 18.5 and 19.0 days) were made using 20 gauge 2.5cm (1.0 inch) hypodermic (Becton Dickinson), 22 gauge 2.5cm short bevel (Monoject, 8881250230), and

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23 gauge 3.2cm (1.25 inch) hypodermic needles.

The effect of pre-set egg storage time was investigated using eggs from the same SPF leghorn flocks collected over three or four day periods. These eggs were stored in the same conditions and incubated simultaneously in the same incubator.

The effect of improper egg rotation was tested by incubating eggs simultaneously in the same incubator and removing one tray (eggs left in upright position) from rotation for 18 hours on day 10 of incubation.

Differences in the in ovo site of injection resulting from incubating in a small scale research incubator (GQF Manufacturing,

Model 1502) or a digitally controlled commercial style incubator (Jamesway 252) were determined. Once injected, embryos were examined for dye location as described below.

● **Egg necropsy for dye location.** Embryos were euthanised and eggs dissected by removing the shell covering the air cell and carefully removing the air cell membrane to expose the embryonic compartments.

Dye location was determined to be air cell (AC), allantoic fluid (ALL), amniotic fluid (AM), or intra-embryo (EM). EM injections were further subdivided into subcutaneous (SQ), intramuscular (IM), and intra-abdominal (IA).

For ease of discussion, the undesirable injection sites of ALL and AC are presented as a combined percentage (ALL/AC). Yolk sac injections were very rare and are pooled with ALL/AC percentages.

Eggs classified as infertile, early dead, middle dead, malformed, or malpositioned (upside down) were removed from the data as these eggs would not be expected to hatch normally.

● **Embryo development stage determination.** Simultaneous with dye location, the developmental stage of the embryo was scored based on four observations: external pip (0, 1), internal pip (0, 1), head under the right wing (0, 1) and degree of yolk absorption (1-4).

The developmental stage score for each embryo ranges from 1-7 with 7 indicating the most developed embryo. All statements of significance are at $p \leq 0.05$.

Results and discussion

The objective of commercial in ovo vaccination is to safely and uniformly vaccinate every viable embryo. To accomplish this objective, a commercial in ovo vaccination device must administer vaccine to sites that result in the greatest efficacy for the vaccine delivered.

Research has shown that delivery of vaccine to the amniotic fluid and embryo body, both of which reside in the amniotic sac, are optimal sites for vaccination.

The Inovoject system was shown to deliver dye to these preferred sites (amnion and embryo) in 98.4% of eggs at 18.0 days of incubation, 99.6% at 18.5 days of incubation, and 100.0% at 19.0 days of incubation (Fig. 1).

Developmental stage scores increased as broiler embryos aged from day 18.0 to 19.0 of incubation (Fig. 2) and embryo age was a significant predictor of developmental stage score. Inovoject system site of injection was significantly correlated with embryo maturation as defined by the developmental stage score (Fig. 3).

As developmental stage scores increased, the frequency of ALL/AC injections decreased and the number of embryo body injections increased (Fig. 3). ALL/AC injections occurred most frequently in embryos that had a developmental stage score of 1 (5.79%), next most frequently in those that scored a 2 (1.62%), at a low rate in those that scored a 3 (0.45%) and none in those that scored a 4 or higher.

Embryos that scored a 1 or 2 had dye delivered to the amniotic fluid at a frequency >92%, while those that scored a 4 or higher had dye delivered to the embryo body >70% of the time (Fig. 3).

These data suggest that methods to measure embryo maturation can be used to predict Inovoject system site of injection frequencies in the late stage chicken embryo.

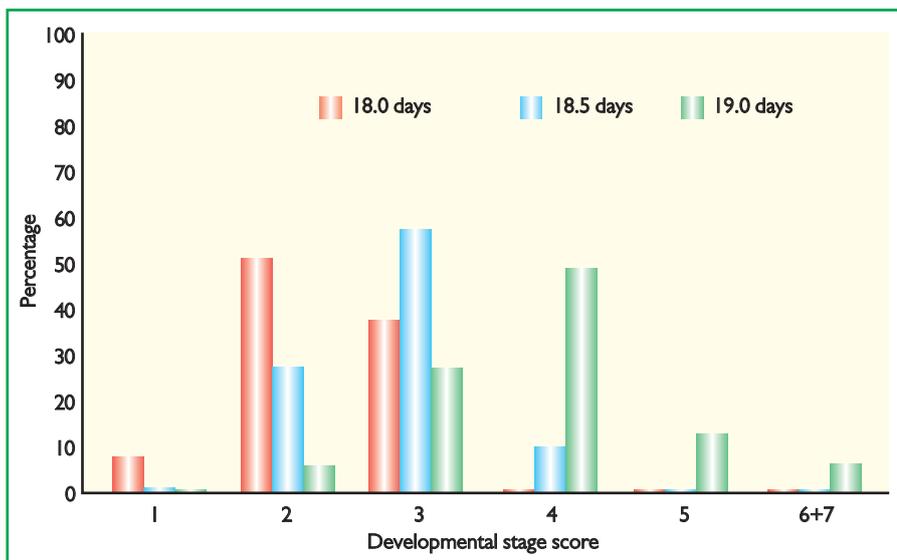


Fig. 2. Distribution of the developmental stage course of commercial broiler eggs at 18.0, 18.5 and 19.0 days of incubation. Embryo incubation age was a significant (P 0.05) factor in determining developmental stage score.

Information of this nature can be used to optimise in ovo vaccination programmes in commercial situations.

The goal of small scale in ovo vaccine research is often the same as that of commercial vaccination, to safely and uniformly vaccinate every viable embryo.

By necessity, small scale in ovo research trials are done using manual injection techniques and often use SPF leghorn breeds rather than broilers.

Embryo age and developmental stage scores of broiler and SPF leghorns embryos used in manual injection studies were significant factors in explaining site of injection frequencies when using a 20 gauge 2.5cm hypodermic needle.

As with Inovoject system data, embryos with lower developmental stage scores (1-3) had a greater chance of being injected in the ALL/AC, while embryos with higher scores (>4) were much more likely to be injected in the embryo body.

Both genetic crosses of SPF leghorns examined at 18.0 and 18.5 days of incubation had mean developmental stage scores that indicated they were approximately 12 hours behind in their development when compared to broiler embryos simultaneously incubated in the same incubator.

The slower development of the SPF leghorn embryos used in these studies certainly contributed to the higher frequency of ALL/AC injections in this breed when compared to broiler embryos.

Because of the many different sources and genetic crosses of SPF leghorn embryos available worldwide one would expect variability in development rates between crosses, which one would expect to impact in ovo site of injection when using manual injection techniques.

SPF leghorn embryos stored for nine days prior to set had significantly more ALL/AC injections and a lower developmental stage score than did embryos stored for either six

or seven days. Improper incubation conditions (no rotation on day 10) resulted in significantly more ALL/AC injections in manually injected SPF leghorn embryos.

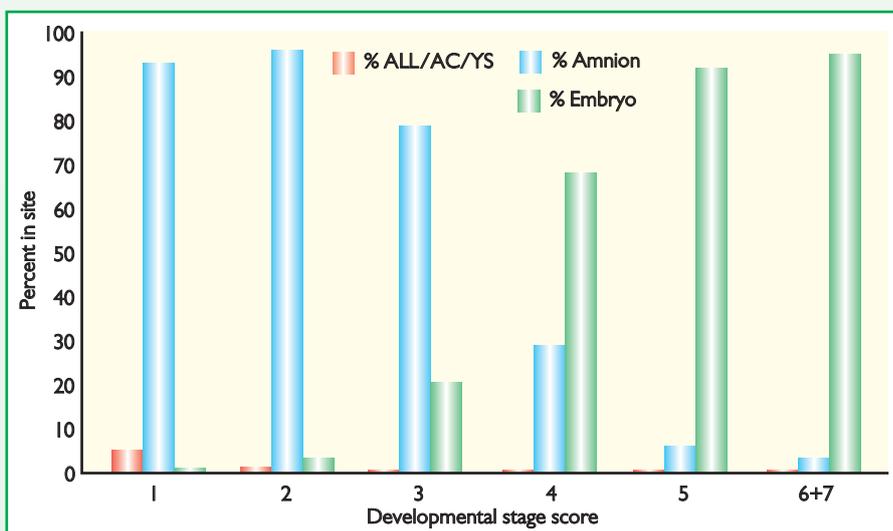
In addition, SPF leghorn and broiler embryos incubated in a small research incubator had a significantly higher number of ALL/AC injections than did same flock embryos incubated in a digitally controlled commercial type incubator.

These data suggest that pre-set storage of greater than seven days or improper incubation conditions can result in an increase in the number of ALL/AC injections.

This is most likely due to suboptimal conditions for embryonic development resulting in less mature embryos on day 18 of incubation.

Researchers doing in ovo experiments should pay special attention to incubation equipment and practices.

Fig. 3. Percent of sites injected by the Inovoject system within developmental stage score of broiler embryos on day 18 of incubation. Developmental stage score was a significant (P 0.05) factor in determining site of injection. ALL/AC/YS = allantoic sac, air cell, and yolk sac injections combined.



Another important contributor to in ovo site of injection is needle length and gauge.

Use of needles of the same length (2.5cm) but of a different gauge resulted in significantly different frequencies of ALL/AC injections.

The 22 gauge 2.5cm short bevel needle vaccinates approximately 2.0mm deeper than the 20 gauge 2.5cm needle, resulting in significantly fewer ALL/AC injections.

Use of a 23 gauge 3.2cm needle decreased ALL/AC injections, but greatly increased the number of intra-embryo injections.

The results of the research reported herein can be explained in a large degree by the developmental maturity of the embryo at the time of injection.

On the 18th day of incubation, the body of a normal embryo is increasing in size and begins to push the air cell membrane upwards.

This upward movement forces the allantoic fluid to the sides and small end of the egg, while the total volume of allantoic fluid is rapidly reduced due to water resorption by the embryo.

As the embryo ages on day 18 of incubation it becomes more difficult to inject dye (or vaccine) into the ALL/AC, while becoming easier to inject directly into the body of the embryo.

In ovo application of vaccines or other biologics needs to be precise for the best chance of success.

In commercial situations this means understanding how various factors affect embryo maturation at the time of in ovo vaccine application.

In research laboratory situations, practices and equipment vary much more than in commercial situations.

It is necessary for researchers to understand the factors that affect embryo maturation and site of injection and strive to control these so that they can accomplish their experimental goals. ■