

# In ovo vaccination for disease prevention

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Twenty years ago in ovo vaccination was a new idea being tested in research laboratories of the United States Department of Agriculture (USDA). Today, in ovo vaccination is being used in commercial applications worldwide throughout the poultry industry.

While the animal health industry has developed a variety of treatments for the prevention of poultry diseases, these treatments are not always administered to the birds in ways that ensure effective and consistent results.

Conventional application has been post-hatch, through feed and drinking water, via a spray that treats the birds through their mucus membranes or hand vaccination. Such treatments require multiple post-hatch vaccinations and field boosts, involving costly guesswork. The result is inefficient, inconsistent vaccine delivery.

The laboratory concept of 'in the egg' vaccination initially used Marek's disease (MD) vaccine and has been expanded into a commercially applied technology platform that is capable of placing several antigens and compounds simultaneously into over 50,000 eggs per hour.

In ovo vaccination enables downstream process improvements in efficiencies such as high speed separation of chick and unhatched eggs, rapid placement or reduced time from hatcher to farm, targeted precise therapeutic intervention and reduction of handling stress on the birds.

## The machine used to administer in ovo vaccination.



Flock age (weeks)	Day of transfer	Number of eggs	Sellable chicks	Hatch (%)	Cull (%)	Net (%)
<31	17	422,676	342,784	82.82	1.75	81.10
	18	421,668	351,546	84.64	1.27	83.37
31-49	17	2,276,424	1,923,148	85.79	1.31	84.48
	18	2,454,048	2,112,552	87.17	1.08	86.08
>49	17	1,270,116	903,824	72.63	1.47	71.16
	18	1,227,708	907,155	75.09	1.20	73.89

**Table 1. Evaluation of embryonic age at the time vaccination based on data collected from a commercial hatchery during a four month period.**

In ovo vaccination offers many advantages over other treatments, such as:

- Earlier immunity – an earlier exposure to various vaccines improves the bird health and disease resistance at the time when they are placed on the farm.
- Uniform delivery – in ovo vaccination allows an automated and uniform process for delivering consistent volumes and concentrations of vaccines to every bird when compared to the previously used subcutaneous method of vaccination at day of hatch.
- Fast delivery – time is reduced from hatcher to farm with vaccinations after hatching being eliminated.
- Reduced stress – birds are less stressed when vaccinated in ovo versus handling and injection after hatch.
- Reduced labour costs – a reduction in the need for the labour associated with day of hatch subcutaneous vaccination is immedi-

ately realised. Compared to the day of hatch vaccination method there is a reduction in labour required to vaccinate the embryos.

- Future products – new products and vaccines have recently been marketed and more are being developed that will effectively control diseases when applied in ovo.

## Site of injection

The process and technique used to administer in ovo vaccines is critical. Delivering vaccine to an incorrect in ovo site can lead to an ineffective vaccination, thus reducing benefits normally seen by vaccinating in ovo.

The measure of success of in ovo applications and vaccine efficacy evaluations depends on not only the manner in which the vaccine is applied, but the timing of the injection in relation to the stage of embryo development and the exact in ovo site of injection (in ovo route) in the developing egg.

An in ovo injection can access five different areas of the egg during late stage incubation. These areas include the air cell, the allantoic sac (waste compartment), the amniotic fluid, the body of the embryo and the yolk sac.

Each area represents a distinct route of vaccine administration to the embryo and this in turn represents distinct types of vaccine and antigen presentation to the avian immune system. Additionally, these in ovo compartments are changing quickly during the 'safe' window of injection timing.

Segregation of waste and nutrients is critical to the survival of the embryo during incubation. Each area within the egg performs different functions during develop-

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ment and the respective fluid dynamics and composition in each area differ. Placement of vaccines and therapeutic compounds into the various areas within the egg may enhance or limit the embryo's response to these compounds.

Research completed at the University of California at Davis showed that the Marek's disease vaccine was not effective with placement of the vaccine on the air cell membrane or when the vaccine was placed into the allantoic sac. The highest efficacy was found in the amniotic fluid with a 94.4% protective index and in the embryo body with a 93.9% protective index (Fig. 1).

The vaccine must be injected deep enough into the egg to be delivered into the amnion, which includes the amniotic fluid and embryo body. When delivered to the amniotic fluid, a substance is imbibed (swallowed) and breathed into the respiratory tract by the embryo prior to hatching.

As the embryo matures and begins the hatching process (internal and external pipping), more embryos in the population are injected in the right breast area.

Embryo body delivery is normal; however penetration too deep into the egg at any stage of development can cause excessive trauma to the embryo. If the vaccine is not injected deep enough, it will be delivered onto the air cell membrane or into the allantoic sac and, therefore, the vaccination will be ineffective. For these reasons, precision is needed to obtain the optimal site (in ovo route) of vaccine delivery.

## The process

The application of egg injection technology is not simply just a matter of delivering vaccines to the egg. The technique must be sterile and controlled and the delivery must be made to precise locations within the egg. Aspects of incubation must be coordinated with the timing and technique of egg injection

to maximise hatchability and vaccine efficacy.

Each hatchery and incubator type may have specific criteria that need to be coordinated with injection and transfer timing to maximise the potential benefits.

The optimum time to inject eggs, developmentally, is the physiological stage between when the stalk of the yolk sac is beginning its ascent into the abdomen and the head is tucked under the wing up until external pipping is initiated. In general, the 'window' for safely injecting the egg is from day 17 and 12-14 hours of incubation to day 19 and 2-4 hours of incubation.

Egg injection on 'early day 17' has been shown to reduce hatchability approximately 1-2% when compared to injection on 'day 18' (Table 1).

## The future

The popularity of the in ovo vaccination machine is spreading worldwide.

Currently in ovo vaccination machines are being used in over 90% of the United States broiler hatcheries and are growing rapidly in Latin America and Europe.

Exciting prospects for disease control and hatchery automation are on the horizon. In the near future it is likely that the technology will include diagnostic capabilities such as determining the sex of the embryo prior to hatch. Other possibilities include the ability to combine different vaccines and better control respiratory diseases.

The future for in ovo vaccination has never been better as various poultry vaccine producers are developing vaccines specific for in ovo applications.

There are a new generation of products on the horizon that only in ovo vaccination make possible. These may require new techniques for vaccinating in ovo, but that will just lead to new opportunities as the methodology of in ovo vaccination is improved. ■

**Fig. 1. Protective indices for different in ovo sites of MD vaccine HVT/IBI administration (Wakenell, et al, avian diseases 46:274-280, 2002).**

