



*in ovo*  
**IN ACTION**

## VACCINATING EGGS

It is generally acknowledged that effective disease prevention is essential for the maintenance of flock health and performance in modern, intensive poultry production. By definition, preventative strategies need to be applied before exposure to the disease threat. Healthy chicks are more likely to lead to healthy flocks, so the hatchery is a natural focus for disease prevention.

For poultry, the earliest possible opportunity to help protect a bird against disease is when it is still in the egg. Several decades ago, this concept led scientists to develop new types of vaccines and delivery devices, which would enable immunity to develop *in ovo*. Since then, *in ovo* technology has become a standard fixture in larger hatcheries around the world. In the US alone, over 90% of broilers are now vaccinated *in ovo*.

The original development work was based around the administration of a vaccine against Marek's Disease (MD), but since then the number of commercially available *in ovo* vaccines has increased, and continues to grow. It is worth noting that only those vaccines that are specifically developed and approved for *in ovo* delivery should be used in BioDevices.

*In ovo* technology has been available for over 20 years and is now tried and trusted. Research has defined the best time to vaccinate embryos in the egg and the best place to deliver a vaccine in order to stimulate the strongest immune response. Both of these factors are critical to the use and design of *in ovo* devices.

The benefits of *in ovo* vaccination can be considered in two broad categories: flock health and hatchery efficiency. Early immunity means that birds get the best possible start when they are transferred to the grow-out farm. The fact that chicks do not have to be subcutaneously injected on day of hatch reduces the amount of handling and associated stress, and also means they can be transferred out of the hatchery more quickly.

As well as promoting flock health, *in ovo* vaccination can also improve efficiency within the hatchery. BioDevices, such as the Embrex® Inovoject® from Zoetis, can vaccinate up to 1.5 million eggs per week, with significantly fewer staff than would be required for subcutaneous vaccination. *In ovo* technology not only allows for greater throughput, but also saves on labour costs and staff issues associated with manual vaccination.

Since the launch of the Embrex Inovoject m from Zoetis, smaller hatcheries can also benefit from *in ovo* technology, even if they don't have the throughput or space to justify a full-sized device. This new semi-automated device is worked by two operators and can vaccinate 12-20,000 eggs per hour.

But *in ovo* technology is not limited to vaccination: additional devices that can further enhance hatchery performance are also available. For example, by applying candling technology, it is possible to identify non-viable eggs and thus avoid administering vaccine unnecessarily – further reducing costs.

*In ovo* vaccination has a whole range of benefits for hatcheries, large and small, that are looking to enhance their business performance in an increasingly competitive market.

To learn more about *in ovo* technology, visit [www.embrexbiodevices.com](http://www.embrexbiodevices.com)

## THE EARLY BIRD

There is an old English proverb: the early bird catches the worm. The benefits of early intervention are certainly relevant to modern poultry production, especially with regards to disease control. Many hatcheries routinely vaccinate chicks on day of hatch in order to help them start developing immunity against common diseases as early as possible. Some are starting to vaccinate their birds while they are still in the egg to get an even greater head start.

In ovo vaccination is usually carried out between day 17.5 and day 19.2 of incubation - with zero hour being the normal egg set time. Studies have shown that this is the optimal 'window' in order to maximise hatchability and subsequent chick performance and immunity during grow-out. In fact the optimal time to vaccinate in ovo is based on the physiological development of the embryo, rather than on the length of incubation, and is optimal from when the stalk of the yolk sac begins its ascent into the abdomen and the head is tucked under the wing until the time that external pipping starts. The exact timing can be adjusted from hatchery to hatchery to ensure the best results.

In ovo vaccine is delivered into the amniotic fluid or into the right breast of the embryo, depending on the stage of development. Before the bird hatches it begins to breathe and ingest the amniotic fluid and so vaccine is taken into the gut and respiratory tract, and absorbed internally by the mucosal surfaces. The gut, of course, contains the bursa of Fabricius, which is the prime immune organ in birds.

As a result, the vaccine is able to initiate an immune response before the chick is hatched, giving it an early advantage over chicks vaccinated on day of hatch. Studies have confirmed that vaccine has a protective index of 94.4% against Marek's disease when delivered into the amniotic fluid, and 93.3% when injected into the embryo body (Wakenell et al. 2002).

It is known that maternal antibodies (IgG) have the potential to interfere with the efficacy of vaccines given within the first few days after hatch. Samples of yolk, blood (serum), amniotic fluid and washings from the respiratory tract of broiler embryos and day-old chicks confirmed that IgG antibody levels peak between day one and day three post-hatch (Data on file 2009). Amniotic fluid and about 30% of respiratory tract washings from the embryos had low levels of pathogen-specific IgG. These results suggest that by vaccinating in ovo around day 18 there is an opportunity to stimulate immunity before the steep rise in circulating maternal antibodies occurs post-hatch. It has been suggested that this low concentration of maternal antibodies in the embryo at day 17/18 may help to explain why in ovo vaccination is so successful.

Direct comparisons between in ovo and subcutaneous vaccination on day of hatch in commercial hatcheries have confirmed that in ovo helps provide better vaccine coverage and earlier protection against Marek's disease (Barbosa et al. 2013). The use of in ovo vaccination also allows chicks to be transferred out of the hatchery with no additional delay or stress as a result of subcutaneous vaccination. The result is a better early performance. So it seems the early birds really do get the worms.

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## DESIGNED TO DELIVER

The principle behind *in ovo* vaccination is simple and compelling: vaccinate embryos before they hatch so that they start to develop immunity to disease as early as possible and with less interference from maternal antibodies. Designing a device that can successfully deliver the correct dose of vaccine, in the correct site, time after time, to tens of thousands of eggs every hour, is more of a challenge.

The world's first commercial *in ovo* BioDevice, the Embrex® Inovoject®, was launched over 20 years ago. The design is based on a number of key features which are critical to successful *in ovo* vaccination.

### ● Adaptability

Eggs vary in size and shape, but an effective *in ovo* BioDevice needs to be able to deliver vaccine consistently in the correct site to a wide range of different eggs. The Embrex Inovoject, for example, overcomes this issue by having independent injection heads which can adjust in three dimensions to fit each individual egg. When the flat is in position within the device, the heads descend and a blast of compressed air is used to locate each tooling cup individually in the right position on its egg, perpendicular to the shell surface. So even if the egg has been leaning to one side during incubation, the injection tool is still aimed at the centre of the egg interior.

### ● Consistent shell penetration

The shell must be punctured in order to allow access for vaccine delivery. A needle designed to inject the embryo is by definition not designed primarily for piercing the shell. The Embrex Inovoject overcomes this problem by using a needle-within-a-needle design. The outer sleeve punches a hole through the shell, allowing the vaccinating needle to then enter the egg and deliver the vaccine. The tip of the outer needle has the optimum angle for piercing the shell with minimal risk of it cracking or shell debris being transferred inside. Its robust construction means that millions of eggs can be pierced without it needing to be replaced frequently.

### ● Accurate site of injection

Again, needle design and size is very important. The Embrex Inovoject uses a 20 gauge needle with a 45 degree straight cut bevel tip - with an opening of less than 1 mm. Studies have shown that this design, combined with the floating head system, delivers vaccine into the correct site with 95% accuracy (Williams & Hopkins, 2011).

### ● Effective needle sanitation

The success of *in ovo* vaccination depends on good sanitation which minimises the risk of contaminants being transferred from one egg to the next. Here the dual needle design of the Embrex Inovoject once again comes to the forefront as it allows sanitising fluid to be automatically flushed down the outer needle and over the injection needle between each injection.

*In ovo* vaccination is a tried and trusted way of increasing hatchery efficiency and chick health, and the design and functionality of the BioDevice is critical for its success.

Williams CJ, & Hopkins BA. Poultry Science 2011; 90:223-226.

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## IN OVO VS SUBCUT

Subcutaneous vaccination of chicks on day of hatch has a number of inherent drawbacks. The process is labour intensive and in high throughput hatcheries requires large numbers of trained staff. The risk of inconsistent vaccine delivery due to worker fatigue or just simple human error is ever present – as is the potential for work related injury.

Injecting chicks by hand is also time consuming and delays the transfer of chicks out of the hatchery and into the grow-out facility. Such a delay in the establishment of a feeding and drinking routine has the potential to adversely affect subsequent bird performance.

Research has shown that chicks subjected to 18 and 36 hours of fasting after placement, corresponding to 30 and 48 hours post-hatching fasting, had lower biometrical values for small intestine (length, weight, and size; villus height; and crypt depth) than chicks fed immediately after placement (Gonzales E *et al.* 2003). Likewise the stress associated with handling and subcutaneous injection has the potential to adversely affect the subsequent performance of the birds.

For hatcheries that want to increase their capacity and the quality of their product, the answer is *in ovo* vaccination. Faster, more consistent and less labour intensive, this form of vaccination is almost universally used in the larger hatcheries in the USA. But how does it compare to subcutaneous injection as a means of delivering effective vaccination?

Studies comparing *in ovo* and subcutaneous delivery of Marek's disease (MD) vaccines were presented at WVPA 2013 in Nantes, France (Barbosa T. 2013). Incubated broiler eggs from commercial hatcheries in Brazil were given an HVT vaccine using Embrex® Inovoject® (Zoetis) at E18 or E19, or manually at one day of age using sc pneumatic vaccinators.

Real-time PCR (polymerase chain reaction) testing of feather pulp from birds at 21 days of age showed that 95% of *in ovo* birds were HVT positive compared to 66.5% of those injected subcutaneously. Furthermore, the range of positive samples was 85-100% for *in ovo* compared to 10-100% for subcutaneously injected birds.

A second study confirmed the less predictable response to subcutaneous vaccination, with 65-100% HVT-positive samples compared to 85-100% for *in ovo*. The results confirmed that *in ovo* vaccination can produce a more consistent response and so is a more reliable method for protecting birds against Marek's disease than manual, subcutaneous injection.

*In ovo* biodevices can vaccinate thousands of eggs per hour with incredible consistency using just a few operators. In the case of the Embrex Inovoject from Zoetis, that means vaccinating up to 1.5 million eggs per week at 95% accuracy (as tested in a commercial hatchery in the US) (Williams & Hopkins 2011). Even smaller hatcheries, with lower throughput or less floor space, can now benefit from improved efficiency thanks to the introduction of the smaller, semi-automated Inovoject m – which can process between 12,000 and 20,000 eggs per hour.

The days of subcutaneous vaccination could be numbered.

Gonzales E *et al.* Poultry Science 2003, 82:1250-1256.

Barbosa T. Efficacy and Marek's disease protection comparisons between different vaccination methods. Presented at WVPA, Nantes, France. June 2013.

Williams CJ & Hopkins BA. Poultry Science 2011; 90:223-226.

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## ADDED VALUE

Since the first commercial *in ovo* vaccination device was launched in 1992, a number of additional technologies have been developed which has further enhanced its performance. These 'add on' technologies are now available as optional extras for some *in ovo* devices.

Candling technology, which uses light transmission through the egg to determine whether the embryo is viable or not, is the basis for two systems which can be added to the Embrex® Inovoject®, from Zoetis.

The first is a candling device that can be used either with the Inovoject or as a standalone piece of equipment. The Embrex® Egg Remover® identifies infertile or early dead eggs and automatically removes them from the flat, discarding them to the side of the device. By removing non-viable eggs, the device can be used to reduce the main source of nutrients for the growth of fungi, such as *Aspergillus*, in the hatchery environment. Removing non-viable eggs cleanly and efficiently can lead to hatch benefits, including healthier chicks and lower instances of field mortality. Sanitation is also improved thanks to quicker post-hatch clean-up from fewer broken eggs, and the device also helps to reduce disposal cost by segregating hatchery by-products.

The second option to identify non-viable eggs is the Embrex Inovoject Vaccine Saver®. This device, which is incorporated into the Inovoject vaccination tooling, works in conjunction with the Egg Remover device. It accurately distinguishes live from infertile or dead embryos, and delivers vaccine exclusively to viable eggs; eggs identified as non-viable pass through without being touched. In practice, the average number of infertile eggs is approximately 10% (Zakaria AH, et al. 2005). The Vaccine Saver device therefore saves vaccine, and money, by making sure that only viable eggs are vaccinated. With the Embrex Inovoject and Vaccine Saver technologies working together, hatcheries can see marked savings in vaccine costs and unmatched improvement in vaccine delivery.

A key advantage of these technologies compared to traditional candling systems is that they provide whole egg population evaluation, as opposed to limited sampling. Each egg is individually verified using the pulsed light technology, and all non-viable ones are removed. This also assists in accurate scheduling of bird placement, saves valuable vaccine doses and enables better hatchability prediction.

The sexing of chicks after hatch and before they are transported to the grow-out facility is a critical point in production. The Embrex Chick Counter speeds up the counting of chicks once they have been sexed and thus reduces labour costs. It reduces the need for labour intensive recounts, improves processing time and provides quality control box count for high speed lines. By eliminating the need for manual recounting, Embrex Chick Counter also reduces the stress associated with getting chicks ready for transport.

Along with *in ovo* vaccination, these devices are helping to automate and increase the efficiency of hatcheries around the world.

Zakaria AH et al.; Oviposition Pattern, Egg Weight, Fertility and Hatchability of Young and Old Broiler Breeders; Poultry Science 2005; 84:1505-1509

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## KEY QUESTIONS

Choosing an in ovo device can be a daunting task. There are a lot of factors to consider, including the performance, build quality and reliability of the device, and not forgetting the reputation and support provided by the supplier. To make the decision a little easier, here are some key questions to ask before choosing a device.

### **Do I have a sufficient number of eggs to process to get a good return on my investment?**

Professional suppliers will have a formula which will tell you if your hatchery could benefit from the introduction of an in ovo device. The company may even have a smaller device option, such as the Embrex® Inovoject® m (Zoetis), which means that hatcheries with smaller throughput can benefit from the technology.

### **Do I have enough space to fit an in ovo device?**

Good suppliers will be happy to carry out a site survey to make sure that the buildings, space distribution, storage facilities, utilities (water/electricity), ventilation, air compressor capability, etc are suitable for installing and operating an in ovo device. Again, smaller hatcheries with limited floor space may still be able to use the smaller, semi-automated Inovoject m, which can be wheeled in and out of the vaccination area and stored when it is not being used.

### **Is my hatchery environment hygienic enough for successful in ovo vaccination?**

This is an important consideration because puncturing the egg shell in the wrong environmental conditions could greatly increase the risk of contamination and failure. Before installing one of its Embrex BioDevices, Zoetis undertakes an environmental assessment to check the level of contamination. Samples are taken from around the hatchery and analysed for the prevalence of fungi and bacteria, but especially for Aspergillus moulds. The survey results determine what work, if any, needs to be done before an in ovo device can be installed and operated successfully. This need for a low contamination risk with in ovo has been one of the driving forces behind the improved hygiene that has been seen in US hatcheries in recent decades.

### **Will the supplier provide suitable training for staff?**

Once the installation has been completed, it is essential that staff are properly trained and on-site support provided during the first few weeks to iron out the inevitable teething problems and optimise operation.

### **Does the in ovo supplier provide good servicing and technical after sales support?**

The aim is to minimise hatchery downtime and make sure any stoppages are dealt with as quickly as possible. Zoetis provides the customer the option to lease the in ovo device which includes a comprehensive, world-class service support. Zoetis operates a rapid call-out support system and makes regular, scheduled maintenance visits to its devices in the field. The company also leaves a stock of common spare parts in the hatchery, so if something happens, parts can be replaced and the device can be working again with minimal down time. It is also important to have access to technical/veterinary advice not just on the device, but also on the vaccines that it is going to deliver.

This is the last article in this series.

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