Optimal timing for in ovo vaccination – understanding embryo development

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n ovo vaccination is currently the standard procedure for hatchery applied Marek's disease vaccine in the Unites States. The laboratory concept of 'in the egg' vaccination has been expanded and developed into a commercially applied technology platform (Embrex Inovoject System) that is capable of placing antigens simultaneously into over 40,000 eggs per hour. Globally more than 15 billion eggs annually are injected in ovo in more than 30 countries.

In order to maximise chick quality, chick performance, and immune status during the grow-out period, it is important to understand the embryonic stage of development. This is critical for timing of egg injection as measured by perfect hatch following in ovo application.

Egg injection too early can reduce percent hatch by increasing the number of late dead and cull birds, as well as an increase in the incidence of missed vaccination. Vaccination too late will create problems for vacuum transfer and increased loss of hatch due to egg breakage.

The precise location of in ovo vaccine deposition is a critical determinant of vaccine efficacy and embryo safety. Delivery of vaccine to an incorrect in ovo site can lead to ineffective vaccinations that erode the



Fig. 1. Accessible embryonic compartments during in ovo vaccination.

benefits typically associated with in ovo technology.

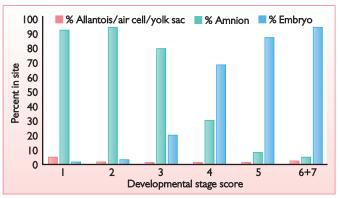
The in ovo route is complex due to the five embryonic compartments that the needle can potentially access during the in ovo vaccination process: the air cell, allantoic sac, amniotic fluid, embryo body, and yolk sac (Fig. 1).

To ensure that the in ovo vaccination system will constantly target the correct site of injection inside the egg there are several factors associated with the egg, the incubation process and the embryo development stages that should be considered in order to maximise the flock immunisation.

Embryonic development

Using a proprietary classification system based on seven points depending on the embryo structures present at the evaluation moment, we found that embryo stages 3 and

Fig. 2. Influence of embryo developmental stage score on site of injection.



4 will ensure a maximum correct site of injection (Fig. 2).

The embryo development stage is a function that is closely related with the incubation time and can be influenced by the incubation equipment.

Some of the most uniform distribution curves in connection with the embryo development stage were observed when the transference was made at 18.5 days of incubation (Fig. 3).

Given the same amount of incubation time, leghorn type birds present a lower embryo development score compared with broiler type birds.

On the other hand, eggs from younger breeder flocks (<48 weeks of age) at 18.5 days of incubation have a lower embryo development score compared with the embryos from older broiler breeders (58 weeks of age) and the percentage of injections in the amnios compartment are higher in the younger flocks, meanwhile in the older flocks the percentage of injections in the body of the embryo are higher.

When a transference was made at 18 days of incubation using SPF leghorn type embryos, the increased egg storage time (7, 8 and 9 days) had an impact on the embryo development stage scores (DS) distribution with significantly increased amount of embryos on DS=1 in the 9 days group, which increase the risk of delivering the vaccines in the wrong site.

At 18-19 days, some but not all of the maternal antibodies in the yolk

have been absorbed by the embryo; full maternally-derived immunity does not develop until a few days post hatch.

If a live vaccine is given to the embryo during this 'window', then the virus can replicate without too much interference from maternal antibodies and thus trigger a good immune response; at the same time the embryo has enough maternallyderived immunity to protect it from developing disease as a result of being vaccinated.

The result is a chick which has the earliest possible immune response and thus protection against disease when it moves into the growing environment.

Final comment

Knowing that the in ovo vaccine's efficacy depends on the embryo compartment where it is delivered, the embryo development stage influences the frequency of which the embryo compartment is injected and that the interactions among type of incubator, incubation time, flock age, bird type and egg storage time impact the embryo development stage; the optimal time for in ovo vaccination has been estimated between 17 days and 12 hours to 19 days and 4 hours of incubation when the embryo has a developmental stage score between 3 and 4 and the embryo is in the position to hatch.

Fig. 3. Embryo developmental stage score distribution curves and dif-

