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Popular article

In Ovo Sexing of Chicken Embryos Using Different Techniques

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The current ethical dilemma with egg production is chick culling of one-day-old male chicks, which kills billions of male chicks each year as part of the production process. The chicks are sexed on the day they emerge from their eggs. The day-old chicks are separated into groups of males and females for chick sexing. After sexing, female chicks are taken to rearing farms where they are kept until they are transferred to laying hen or broiler farms &the majority of male chicks that hatch after being sexed as male are killed.

Gender determination in ovo has the potential to stop the slaughter of billions of male chickens. An estimated 7 billion male chicks under the age of two are euthanized each year. The chicken industry produces more sustainably and with greater animal welfare when in-ovo sexing is used. The first study on the subject which focused on in-ovo sexing on the ninth day of incubation—was released in 2013. A hormone test for the allantoic fluid of brown layer eggs was used in the study process, which was subsequently dubbed bio-marker detection. Using the same methods, Prof. Dr. Einspanier conducted more study. It is noteworthy to remark that, according to Dr. Einspeiner, this in-ovo sexing procedure affects hatchability.

The fluorescence spectroscopy technology was created in 2016, and it analyses extraembryonic blood to determine the sex of the embryo using blood wavelength. By using a PC with a 93% error rate for supervised egg classification, 380 eggs at 3.5 incubation days could have their sex determined. In 2017, a variant of this methodology was investigated. When the methodology for pattern analysis in hyperspectral pictures was studied. Halogen lamps would be used to first candle the eggs. After that, the transmitted light would be gathered by a hyperspectral camera, and a linear discriminant analysis would be used to classify the

eggs. With this technology, 97% of 11- to 14-day embryos may be successfully sexed in utero. 2019 a new methodology was developed, AI-powered imaging. By combining AI and MRI together to perform in- ovo sexing of 12-day-old eggs with 95% accuracy level.

Technologies must be-

- **Invasive or non-invasive**: Some technologies require making a hole in the egg. This is a concern, since it creates a risk for contamination and lower hatchability. Therefore, non-invasive technologies are preferred.
- **High accuracy**: The technology must be very accurate in determining the gender. The accuracy needs to be in line with existing levels of accuracy when sexing chicks after they hatch.
- **The sooner the better**: The sooner the sex can be determined in the egg the better. To address animal welfare concerns, eggs should be sexed before the age of pain sensation. Although scientists disagree about the exact number of days at which pain is felt, it is clear sooner is better.
- **High speed of sexing eggs**: To function in a commercial hatchery, the speed at which eggs need to be sexed must be very high. This is particularly important if sexing happens when eggs have already been in the incubator - to prevent them from losing too much heat. So, technologies need to operate at high speed.

Ongoing initiatives and their approaches

- **Spectroscopic detection**: Spectroscopy is the technique of splitting light (or more precisely electromagnetic radiation) into its constituent wavelengths (a spectrum) in much the same way as a prism splits light into a rainbow of colors. It requires shining a light beam of laser through the eggshell or a hole in the egg. Via image analysis, the gender is determined. Although training the software to be sufficiently accurate requires tremendous amounts of data and therefore time, this technology does offer the prospect of early identification.
- **Biomarker detection**: This form of analysis requires taking a fluid from the egg, mixing it with a biomarker, and analyzing the resulting sample. In most cases, allantoic fluid is withdrawn. The fluid is then used for a test that allows to see the gender. There are several different tests being investigated or developed. Most of these test's work after day 7 or 8.
- **Gene editing**: A few projects are working on a solution using gene editing. The regulatory framework for this is still unclear as is consumer acceptance. On the other

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hand, if it were to be widely accepted, the benefits of such a technology would be that it is non-invasive and provides opportunity for pre-incubation egg sex detection.

Some technologies-

PLANTegg

The PLANTegg process, a PCR-based technique for determining the gender of hatching eggs, is the result of the development and combination of new technology. The hens' distinct gender chromosomes serve as the foundation for this molecular genetic technique. The genetic variations present in the DNA of the allantoic fluid become evident thanks to PCR technology. The PLANTegg process

1. **Access to the Egg**

The hatching eggs are transferred to a robot, which first creates a tiny opening in the hatching egg with the help of a laser beam.

2. **Processing**

A few drops of the allantoic liquid are taken from this opening with a pipette and transferred to test tubes. The allantoic fluid contains genetic material (DNA) which provides enough information for a genetic examination.

3. **DNA Analysis**

The allantoic fluid is combined with a reactive medium and transferred to a PCR machine. There the multiplication of the specific DNA of the gender chromosomes takes place, so that male and female hatching eggs can be determined with high precision.

4. **sorting**

Finally, the female eggs are returned to the hatching process and the male eggs are used for other purposes (e.g. as feed).

Seleggt

The SELEGGT process is a way to prevent chick culling. The scientific approach of endocrinological (hormone-based) gender identification in the hatching egg has been extensively automated with the SELEGGT process and is already being used in practice today. During the process, the nine days incubated hatching eggs are removed from the incubator and checked for fertilization based on sensors. A precision laser opens the eggshell by creating a small hole with a diameter of approx. 0.3mm in the eggshell of all fertilized hatching eggs. A small amount of allantoic fluid is then extracted. The allantoic fluid of female hatching eggs contains estrone sulphate, a female gender hormone. The fluid is extracted non-invasively by means of a precise, electronically controlled suction process. Consequently, it has no negative

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consequences for the hatching egg, as the inside of the hatching egg remains untouched and unharmed.

The fluid sample is now applied to a patented marker outside the hatching egg. The marker reacts to the estrone sulphate with a color change. The hatching eggs can now be distinguished and sorted into male and female gender according to the color change. This way, it is possible to process the male hatching eggs into high-quality feed, while the female hatching eggs are returned to the incubator.

Immediately after the allantoic liquid has been extracted, the hole in the eggshell is sealed with beeswax to minimize the risk of contamination of the inside of the hatching egg.

In Ovo

The in-ovo sexing technology they use is based on biomarkers and is invasive. A sample is taken from each egg, mixed with a biomarker, and examined by mass spectrometry.

Ella is a machine that determines the sex of an egg instead of a chick, ensuring only females are hatched, thereby preventing the culling of male chicks. A fully automated, inline solution, Ella can determine the sex of an egg on day 9 of the incubation period.

Ella takes a sample from the allantois, which is the waste sac of the hatching egg. It analyses this sample for the concentration of a biomarker that allows it to determine whether an egg is male or female. After sampling, each egg is immediately closed using biodegradable glue.

Ella automatically picks up trays from the trolleys, removes unfertilized eggs before sampling and sorts male and female eggs after analysis.

Hypereye

Hypereye is a patented scanning in-ovo sexing technology that uses non-invasive hyperspectral imaging. This is done at day 0, pre-incubation. The technology was developed by Michael Ngadi at McGill University in Canada, with funding from Ontario Poultry Industry Council, Egg Farmers of Ontario and Livestock Research Innovation Corporation.

GA-BPNN based gender recognition algorithm 89.74% accuracy

The genetic algorithm (GA)- backpropagation neural networks (BPNN): a non-destructive method of gender identification of chick embryos at Day4 for integrity of blood vessels in the field of machine vision.

Male embryo: thick main blood vessels with many lateral vessels and few thin branches in uniform distribution.

Female embryo: thinner main blood vessels with fewer lateral vessels and more thin branches

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in irregular distribution

Poultry by Huminn

Gene editing technology regulates activation of Z chromosome by Optogenetic system, uses a blue light that shines through the eggshell at the incubator "Golda chicken" in 2021The genetic change affects only the female parental Z chromosome Male embryo will cease developing in early stage of embryonic & not hatch, eliminating the need for sexing, sorting and culling

CRISPR/Cas9

CRISPR stands for Clustered regularly interspaced short palindromic repeats, is a method of genome editing technology, a powerful tool for live cell imaging of genomic loci to govern the gender of embryonic chicken. eggXYt is a CRISPR-Cas9 technology developed by Israeli Biotech company. Male eggs can be identified by electro-optical scanner & seen by bright yellow color while female eggs aren't affected achieved by labelling Z chromosome of hen with green fluorescent protein

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