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Big Data Enabled Non-Invasive Rapid Sex Detection of Incubated Chicken Eggs

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Commercially produced chickens serve essentially two purposes: “broilers” for meat and “layers” for table eggs. Layer chickens, bred to mature more slowly, can lay commercial eggs within 16–21 weeks after hatching. Male layer chicks do not lay eggs upon reaching maturity and their use is commercially limited, while some are fattened in organic like systems for the meat market owing to lower growth rate and higher feed conversion ratio compared to broiler chicks. Male chicks — billions a year — are macerated in mechanical grinders or suffocated with carbon dioxide or argon gas, usually within a day of hatching. Besides obvious animal welfare and ethical/societal considerations, (shredded) chick carcasses have limited commercial value. European Union legislators currently are drafting a raft of directives and regulations concerning the practice of male chick culling; and EU countries to outlaw chick culling, by the end of 2024 (Bressan et al., 2018). There is an immediate need for development of methodologies for non-invasive determination of the sex of an embryo of chicken egg, using imaging and spectroscopy technologies.

Background

Current commercial, pre-commercial, and experimental *in ovo* techniques for the sex determination of fertilised eggs employ either minimally invasive biomolecular assays (extracting fluid via a small laser-drilled window in the eggshell, for detection of genetic or hormonal biomarkers), analysis of volatile compounds emitted from the eggshell, visible imaging, and reflectance or transmission spectroscopic analysis exploiting molecular optical fluorescence, polarisation, and scattering phenomena, including various combinations of these modalities. Among the biomolecular techniques, the “*Seleggt*” process, which employs a proprietary biomarker to detect the presence of estrone sulphate in the allantois fluid, generally is acknowledged to represent the benchmark of the current state-of-the-art for in ovo sex determination. More speculative approaches include reversal of the developing embryo’s gender through *in-ovo* gonadal manipulation, employing aromatase inhibitors of estradiol synthesis. By combining ELISA with PCR data, an integrated biochemical and genetic information can be explored initially as a two-pronged approach in earlier sex determination of chicken embryos.

Techniques attracting much attention in current research include near-infrared (NIR) Raman spectroscopy and Fourier transform infrared spectroscopy (FTIR) (Hyperspectral Imaging). RAMAN spectroscopy is a multipurpose analytical, reagent-less optical technique that provides a unique spectroscopic fingerprint of biological samples in solid, liquid, and vapor forms. Based on the inelastic scattering of the light that occurs from interactions between incident photos and molecules in the sample, RAMAN spectroscopy can be quite useful as a non-invasive real-time, assessment and online measurement of various biomarkers from eggshell, allantoic fluid, blood vessels and/or the odour volatile micro signatures on the shell surface. Hyperspectral imaging system operating in the visible and Visible-Near Infrared spectrum of electromagnetic radiation

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offers an extremely low noise, high resolution, and rugged structure and as a powerful tool for non-invasive investigation of various biological substances. Unlike routine imaging or spectroscopic methods, hyperspectral imaging offers both spatial and spectral parameters simultaneously. Each position within the sample (fluids, blood or eggshell surface) displays a unique spectral fingerprint of that individual pixel, which is then used to characterize the chemical component.

Sex determination can be achieved through complex data processing of the raw optical signals obtained from the spectral information in a non-invasive manner. This could be practically performed through conventional statistical (regression) analysis or with more advanced machine learning data reduction and feature extraction pre-processing (e.g., principal components analysis) and pattern recognition classifiers (e.g., support vector machines, Mahalanobis distance) that are trained and validated on big data sets acquired from incubated eggs having confirmed post-hatch gender outcomes.

However, to date no endeavour employing the NIR and FTIR based spectroscopic techniques has resulted in a commercially sustainable solution to the egg sexing problem. Besides achieving only subpar performance in overall accuracy, specificity, and sensitivity, the least invasive of the current state-of-the-art optical methods still requires, creating a transmission window (fenestration) of 12–15 mm diameter through to the mammillae layer of the shell, proximal to the external shell membrane, which can affect the incubation or post-hatch development viability of up to 10% of incubated eggs.

Challenge

Three principal attributes are necessary for a viable sex sorting process installed in a commercial egg production line: sex labelling accuracy (> 90%), effective before day 7 — ideally by day 3 — of incubation (after which embryonic pain receptors have formed), and rapid throughput (> 10 eggs per second). Additional, desirable, attributes are for the process to be non-destructive, minimally invasive, and not requiring laser fenestration of the eggshell. Recent reports pointing to usage of MRI (Orbem/Vencomatic partnership) as a non-invasive technique needs scientific validation as there are quite a few challenges and many unknowns surrounding MRI based multiparametric quantitative imaging for a high-throughput and ‘predictive’ capabilities.

Concept and Innovation

Hyperspectral imaging is quicker in providing spectral information in comparison to RAMAN. To overcome concerns for point-based analyses of the bio-chemical signatures from the ambience of the chicken embryos, a combination of techniques allows for best results. By using RAMAN Spectroscopy in conjunction with hyperspectral imaging, not only false positives (unambiguous identification) can be avoided, but also this conjunction offers superior quality biochemical information along with suitability for large area scanning on the egg surface and offers smaller power requirements and better choice for mobile applications and integration. An exhaustive comparative literature review of leading edge research on minimally invasive techniques for chicken egg sexing, together with our own expertise into current progress in the field, leads to conclude that a particular multimodal solution combining Raman spectroscopy and hyperspectral imaging has strong prospects to overcome the hard barriers existing before the perfection of a non-

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invasive in-line process for high reliability and rapid throughput for sex determination of eggs within 3 days of incubation.

Approach

Analysis of multispectral imaging data obtained from the hyperspectral plus Raman scattering hybrid sensor may provide mapping of key biochemical signatures on the eggshell surface as well as several microns below the eggshell, accessing the fluid within the allantoic cavity. By synergistically making use of the quantitative imaging protocols by coupling the analytical data from Raman spectroscopy with the multispectral information, insights can be derived. In addition to spectroscopic analysis and extensive machine learning based algorithm development, biochemical assays can be employed for cross-validation. Through assessing the possibility to combine an ELISA assay of the allantoic fluid with PCR-based genomic evaluation, additional information regarding sexing discrimination can be obtained.

Multiple sex-discriminant biomarkers are, in principle, available for detection by optical and spectrographic methods. Estradiol (E2) — an oestrogen steroid hormone — is secreted by the chicken embryo ovary, indicating development of female chicks. Moreover, DAX1 and FET1 genes are expressed only in female embryos. The Anti-Müllerian hormone (AMH) regresses and inhibits the development of the female reproductive tract (Müllerian ducts) in the male embryo, and the hormones DMRT1 and SOX9 govern the formation of testes. At what stage of incubation these hormones manifest and are available for detection in the allantoic fluid currently is unknown and needs to be explored.

Chick embryo SA node pacemaker cells are established by day 6 of incubation [Bresslan et al., 2018]. It has been speculated that estrogen contributes to an increase in cardiac automaticity and there may be relationship between time points in the formation of pacemaker cells to the gonadal development. This possibility of interrelationship can be explored through biochemical assays and imaging experiments. By attempting to identify, via the multispectral technique, the specific protein biomarkers in either embryonic blood or allantoic cavity fluid that might indicate the presence of pacemaker cells can be found out. Although this data would not provide information specifically on the embryo's sex, one can theorize that pacemaker cells differentiate in chick embryos at a similar stage to gonad development. This information initially can be made use of in assessment using a combination of biochemical/genetic assays correlated with the combined Raman and hyperspectral information to achieve sex identification.

The method for sexing of chicken embryos can then take a *multipronged approach* in collecting and analyzing spectral data that points to these above-mentioned biomarkers and using the machine learning approaches to look for nanomolar to picomolar concentrations of these in the fluid. This will create that much needed shift to less than 6 days, or even 3 days, to determine the sex of chicken embryos.

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