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Non-destructive Classification of Chick Embryos Based on Heartbeat, Body Motility and Growth Using Signal Processing of Near-infrared Light
（近赤外光の信号処理を用いた心拍，運動性および成長に基づく鶏胚の非破壊分類)

## ABSTRACT

Poultry became the largest source of meat consumed in the world when it overtook pig production in 2017. In order to further promote sustainable poultry production systems to feed future generations, the monitoring and sorting of chick embryos rather than waiting to grade day-old chicks would enable higher productivity and more efficient use of resources. In this respect, non-destructive monitoring of chick embryos (hatching time, gender, cardiac abnormalities) during incubation could provide vital management insights for poultry farmers and other stakeholders. Non-destructive identification of hatch-window, gender and cardiac arrhythmias are also promising research areas with respect to promoting animal welfare in the industry. This is because late hatch chicks are a source of many complications in subsequent chick grading and rearing due to their delayed growth and retarded post-hatch performance. Hence, an understanding of the physiological factors leading to late chick hatching, along with early detection of these factors could avoid disadvantageous post-hatch culling.

Besides, there is a gender biased preference in dimorphic birds like chickens where the male is the preferable gender in broiler, and the female in layer strains. For broiler production female chicks are not economically viable due to their lower growth rates compared to male counterparts. Every year more than 7.0 billion day-old layer strain cockerels are culled globally by asphyxiation with carbon dioxide or maceration due to the noted gender biased production preference; raising serious ethical issues and resulting in significant economic losses. Therefore, non-intrusive, early chick embryo sexing before hatching is a crucial issue both from a commercial and ethical (animal welfare) point of view. This has driven increasing research effort and the desire of other stakeholders in the industry to find a solution. On the other hand, cardiac arrhythmia is considered an important cause of cardiovascular disorder in birds. Little is known, however, about arrhythmia during embryonic stages, chiefly because of their relative rarity and difficult of diagnosis. Unfortunately, the electrocardiogram (ECG) method cannot be used to study avian embryos due to the eggshell barrier. Therefore, an alternative method is an important tool if avian or reptilian embryo research in this area is to advance.

To remedy these issues, the study investigated the feasibility of using a near infrared (NIR) sensor together with signal processing to simultaneously monitor embryonic body motility, heart beats both in-terms of frequency and strength, as well as opacity (embryonic growth), towards the end of predicting hatch-window, and detecting gender and cardiac arrhythmia. Broiler chicken eggs (ROSS 308), selected based on size, weight, shell colour, were incubated for these purposes. Embryonic activity signals, based on NIR transmitted LED light and photodiode detection, of individual embryos were measured every 24 hours from day 6 to 19 of incubation. Peak frequencies and the power spectrum from these signals

were extracted using Fast Fourier Transform and numerical integration. The signals were also filtered using a low pass filter to visualize the characteristic signal patterns.

Two types of chick embryo body movements were found throughout the whole incubation period. During the early stage of incubation (day 6 to 8), the movement has a periodic pattern with a characteristic shape that differs among the embryos with a frequency ranging from 0.3-0.8 Hz. In the mid to late stages of incubation, movements were random, and had a lower frequency range from 0.2-0.6 Hz. Body movement strength was highly random, especially after day 10 of incubation. Heart rate during the incubation varied from 3.8 to 4.8 Hz in the period from day 8-19, while heartbeat strength sharply increased during incubation, peaking at day 14 to 16, and then subsequently subsiding. while heartbeat strength sharply increased during incubation, peaking at day 13 to 14, and then subsequently subsiding. While heartbeat was found to be related to hatch window, body motility and opacity were dependent on chick embryo gender during incubation. In terms of cardiac output, the higher the cardiac output the shorter the incubation period (an earlier hatch window). Heartbeat can be used to separate late hatch chicks with an accuracy of about 90%. On the other hand, male embryos during the second half of incubation were found to be more active and heavier than female embryos in-terms of body motility and opacity (growth), respectively. Chick embryo gender can be detected based on body motility strength and growth with the accuracy of 84% before hatching. In addition, we also investigated and interpreted embryo cardiac signals with naturally occurring bradycardia throughout the incubation. We found a normal heart rate (HR) during first half of incubation, but the HR had two frequency components in the mid incubation period and finally a much lower heart rate up until hatching. Early detection of potentially abnormal chicks with cardiovascular defects could significantly contribute to the humane treatment of these embryos and increase production efficiencies by identifying high quality embryos.

These results indicate that the NIR sensor, combined with signal processing, has the potential to non-invasively detect various hatch-groups, gender and cardiac arrhythmia. These techniques will enable poultry hatchery managers to ensure more precise monitoring and to enable sorting of embryos with the aim of obtaining uniform and high-quality chicks. Moreover, this could help to reduce costs by reducing the rearing time of unwanted embryos (space, energy and labor costs), as well as minimizing post-hatch handling for sorting of unwanted embryos. This research could also open many new dimensions of non-destructive research in precision poultry production and biomedical engineering fields such as embryo grading systems, indirect blood pressure measurement in avian and reptile models; cardiovascular drug design research; oxyhemoglobin level estimation in blood and in many other areas in the future.