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Comment

Comment on Jia et al. Exploratory Study of Sex Identification for Chicken Embryos Based on Blood Vessel Images and Deep Learning. *Agriculture* 2023, *13*, 1480

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This is a comment on "Exploratory Study of Sex Identification for Chicken Embryos Based on Blood Vessel Images and Deep Learning" (Jia et al., *Agriculture* **2023**, *13*, 1480) [1].

1. Implications for the Field

The early sex identification of chicken embryos is a scientifically and economically important aim, with important ethical and sustainability implications for poultry production. However, robust, reproducible, and generalizable methods are needed before any technique can be adopted in commercial practice.

2. A Summary of the Original Study

Ref. [1] reported a method for the early sex identification of chicken embryos using blood vessel images and deep learning object-detection models (YOLO-v5 and improved YOLOv7). The model was trained on a dataset of about 4000 vascular images (from approximately 2000 eggs) collected from incubated chicken eggs (day 3 to day 5 of incubation). The model was tested on a dataset of about 800 images from 400 eggs. The imaging of the blood vessels was performed using brightfield imaging (egg candling) through the intact egg. According to the publication, the model achieved high accuracy scores, above 88%, for classifying embryos as male or female based on vascular features, suggesting potential for the practical application of early non-invasive sexing in the poultry industry.

3. Unsuccessful Reproduction Attempt of Sex Identification with Laser Speckle Contrast Imaging (LSCI)

In a subsequent independent study published in PLOS One, Mahler et al. (2026) [2,3] attempted to replicate the core findings of the initial study using blood vessel images recorded by a laser speckle contrast imaging (LSCI) system [4,5]. Deep learning models such as ResNetBiT and YOLO-v5 were used for sex classification. The model was trained on a dataset of about 1100 vascular images (from approximately 800 eggs) collected from incubated chicken eggs on days 3 and 4. The model was tested on a dataset of about 300 images from about 200 eggs. According to the results, the model achieved a consistently low accuracy score, below 60%, for classifying male and female embryos [3]. The authors of [3] report that vascular pattern imaging alone was inconclusive for reliable early (days 3–5) sex identification, contrary to [1]. This suggests that the results of the original study could not be obtained under similar experimental conditions with an alternate imaging

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approach, raising questions about the generalizability and robustness of the original model and dataset.

The data that support the findings of the subsequent study are publicly available from the Zenodo repository dataset at https://doi.org/10.5281/zenodo.20316359. The scripts and codes used for deep neural network analyses can be found from the Github repository at https://github.com/anika24/lsci-egg-sex-classification(accessed on 28 September 2026). Note that the original study [1] does not share its dataset or code, even upon request.

4. Mahler et al.'s Subsequent Study

Mahler et al. performed an independent investigation using 1251 chicken embryos and 2773 LSCI images, with sex determined after incubation through gonadal examination and further validated by PCR. They evaluated two deep learning approaches, ResNetBiT and YOLOv5s-cls, using five-fold cross-validation and reported classification accuracies of approximately 55–57% for embryos imaged on days 3 and 4. These results did not reach the level of statistical significance or practical accuracy required for reliable early sex classification under their experimental conditions.

The study provides important evidence that early sex classification from extraembryonic vascular images (obtained from LSCI) requires further validation. At the same time, because the study employed LSCI rather than the transmission/candling approach used by Jia et al., its results should not be interpreted as a direct replication or concrete disproof of the original findings. The substantially different performance observed under an independent experimental and analytical framework emphasizes the need for additional studies using independent datasets and imaging systems to determine whether the reported classification performance is robust and generalizable across experimental conditions.

5. Comparison Between the Two Studies

There are several differences between the two studies:

- Differences in Imaging Methods: The authors of [3] relied on laser speckle contrast imaging (LSCI), which captures active blood vessels by measuring speckle contrast variations caused by blood flow differences. In contrast, Ref. [1] used egg candling (brightfield imaging), which detects light absorption differences between blood and other background elements. Both imaging methods are optical-based and provide similar, if not identical, representations of blood vessel imaging [4]. While LSCI specifically images active blood vessels, all embryos in both studies were alive and functioning normally. Both imaging methods, egg candling (as in [1]) and LSCI (as in [3]), were compared in detail in Ref. [4] for chicken and quail eggs. The results (Figures 3 and 4 in [4]) show similar, if not identical, blood vessel structures between the two imaging methods, with LSCI having the advantage of being less sensitive to eggshell pigmentation, cracks, and stains. Therefore, the two imaging methods provide equivalent, or at least highly similar, representations of the underlying blood vessel structures.
- Image Quality Comparison: The overall quality of blood vessel images appears to be similar. Supplemental Figures S2 and S3 in [3] provide randomly selected samples of 16 blood vessel images from days 3 and 4 of incubation, which can be visually compared to those in Figures 3 and 4 in [1]. The images in [3] are monochrome grayscale, whereas those in [1] are in color. However, for a green LED light source, typically only one channel (the green one) contributes to relevant blood vessel details [4]. Therefore, both datasets appear to be equivalent regarding image quality.
- Different Egg Types: The authors of [2] used brown eggs, whereas [1] used white Jingfen No. 6 eggs. Biologically, there is no expected difference in embryonic devel-

opment (including sex development) between these two breeds. As demonstrated in Ref. [6], which specifically examined embryonic blood vessel development from day 0 to day 5 of incubation across different chicken breeds and eggshell colors, embryonic development follows the same developmental trajectory during this early incubation period regardless of breed or eggshell coloration. Differences in the developmental trajectory of the vasculature (i.e., blood vessels) start at the onset of the chorioallantois membrane (CAM), around day 4.5. Thus, we consider it unlikely that the difference in egg type alone explains the substantial difference in classification performance, particularly when comparable blood vessel image quality is achieved. Nevertheless, we acknowledge that breed- and egg-specific differences could potentially affect imaging characteristics and therefore should be considered when assessing the generalizability and reproducibility of the reported results.

- Ground-Truth Determination: The authors of [3] established ground-truth sex identification by combining two independent methods: gonad identification and PCR testing from dissection on day 11 of incubation. Meanwhile, Ref. [1] determined sex based on feather color at hatching [7]. The accuracy of the feather method is operator-dependent and can range from 75% to over 95%. The accuracy of gonad identification combined with PCR testing ranges above 95%. Note that in [3], embryos with a mismatch between gonadal sex and PCR-based sex determination (approximately 4% of the samples) were excluded from the deep learning classification.
- Data Availability: We were unable to obtain the dataset from Ref. [1] for direct comparison, despite multiple attempts to contact the authors of the original study or the editorial board of the journal. The dataset from Ref. [3] is publicly available at the Zenodo repository at https://doi.org/10.5281/zenodo.20316359.
- DNN Model Differences: The authors of [1] used YOLO-v5 as the baseline, reporting slightly lower accuracy for YOLOv5 compared to the modified YOLOv7 model. The authors of [3] also used YOLO-v5. Given the small performance gap between YOLOv5 and YOLOv7 in their results, a difference in the DNN architecture alone is unlikely to account for the large discrepancy in accuracy.
- Dataset Size: The authors of [1] used about twice as many eggs and around three times as many images as [3]. Although larger datasets generally contribute to improved model performance, the difference in sample size alone is unlikely to explain the substantial disparity in classification accuracy and binary cross-entropy (BCE) loss reported between the two studies.

6. Concerns About Generalization and Transparency

Repeated attempts to contact the authors of [1] and the editorial office for access to source code and data, or additional methodological details, have not been successful. Such materials are essential for independent studies, particularly in machine learning research where implementation specifics can materially affect results. Without access to the original dataset and preprocessing pipelines, it is difficult to determine whether differences in imaging modality, preprocessing, or model training are responsible for the divergent outcomes.

7. Conclusions

The authors of [3] were unable to accurately determine the sex of chick embryos from LSCI images on days 3 and 4 of incubation using a YOLO-based model (accuracy consistently below 60%). The results are surprising given that [1] reported over 88% accuracy with low loss using YOLO-based models on chick embryo blood vessel LED images from the same developmental stages. The inability to access the data and/or the

code used in [1] furthermore underscores broader concerns regarding transparency and methodological reporting.

On a side note, it was reported that genetic expression and sex differentiation in chick embryos begins around day 4.5 in male embryos and around day 6.5 in female embryos [8], with gradual morphological changes in the gonads becoming observable afterward.

The rationale for using early vascular images for sex identification warrants further clarification. Although deep learning models may identify vascular features that are difficult to distinguish visually, they may also exploit other image-related characteristics.

Finally, if the difference in performance is ultimately attributed to the difference in imaging modality, this would raise an important question regarding what image features are actually being exploited by the sex-classification model in Jia et al., "Exploratory Study of Sex Identification for Chicken Embryos Based on Blood Vessel Images and Deep Learning" [1].

Data Availability Statement: The data that support the findings of the subsequent study are publicly available at the Zenodo repository dataset at https://doi.org/10.5281/zenodo.20316359. The scripts and codes used for the deep neural network analyses in this study can be found at the following Github repository: https://github.com/anika24/lsci-egg-sex-classification (accessed on 28 September 2026).

Conflicts of Interest: The author declares no conflicts of interest.

References

1. Jia, N.; Li, B.; Zhao, Y.; Fan, S.; Zhu, J.; Wang, H.; Zhao, W. Exploratory Study of Sex Identification for Chicken Embryos Based on Blood Vessel Images and Deep Learning. *Agriculture* **2023**, *13*, 1480. [CrossRef]
2. Mahler, S.; Arora, A.; Readhead, C.; Yin, S.; Hari, S.N.; Wang, E.; Moxley, C.I.; Adeboye, A.A.; Dong, Z.; Zhou, H.; et al. Exploring non-invasive sexing of early chick embryos in intact eggs using Laser Speckle Contrast Imaging (LSCI) and Deep Neural Network (DNN). *bioRxiv* **2025**. [CrossRef]
3. Mahler, S.; Arora, A.; Readhead, C.; Yin, S.; Hari, S.N.; Wang, E.; Moxley, C.I.; Adeboye, A.A.; Dong, Z.; Zhou, H.; et al. Detection of non-invasive sexing of early chick embryos in intact eggs using laser speckle contrast imaging and deep neural networks. *PLoS ONE* **2026**, *21*, e0323847. [CrossRef] [PubMed]
4. Dong, Z.; Mahler, S.; Readhead, C.; Chen, X.; Dickson, M.; Bronner, M.; Yang, C. Non-invasive laser speckle contrast imaging (LSCI) of extra-embryonic blood vessels in intact avian eggs at early developmental stages. *Biomed. Opt. Express* **2024**, *15*, 4605. [CrossRef] [PubMed]
5. Readhead, C.; Mahler, S.; Dong, Z.; Sato, Y.; Yang, C.; Bronner, M.E. Automated non-invasive laser speckle imaging of the chick heart rate and extraembryonic blood vessels and their response to Nifedipine and Amlodipine drugs. *Dev. Biol.* **2025**, *519*, 46–54. [CrossRef] [PubMed]
6. Jin, Z.; Kakkar, S.; Chenemilla, D.; Readhead, C.; Bronner, M.E.; Mahler, S. Longitudinal, Non-Invasive Imaging of the Developing Chick Heart, Vasculature, and Chorioallantoic Membrane (CAM). *bioRxiv* **2026**. [CrossRef]
7. Gryzińska, M.; Batkowska, J.; Andraszek, K.; Horecka, B.; Jeżewska-Witkowska, G. Changes in plumage color and patterns in Polbar breed chicks (Polish conservative breed) during their first weeks after hatching. *Eur. Poult. Sci.* **2014**, *78*, 1–10. [CrossRef]
8. Rahman, A.; Syduzzaman, M.; Khaliduzzaman, A.; Fujitani, S.; Kashimori, A.; Suzuki, T.; Ogawa, Y.; Kondo, N. Nondestructive sex-specific monitoring of early embryonic development rate in white layer chicken eggs using visible light transmission. *Br. Poult. Sci.* **2020**, *61*, 209–216. [CrossRef] [PubMed]

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