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This is an author-produced version of a paper published in *Avian and Poultry Biology Reviews* (ISSN 1357-048X). This version has been peer-reviewed but does not include the final publisher proof corrections, published layout or pagination.

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Citation for this version:

Shapland, F; Baggott, G.K. (2006) The effect of static incubation on the yolk sac vasculature of the Japanese quail (*Coturnix c. japonica*). *London: Birkbeck ePrints*. Available at: <http://eprints.bbk.ac.uk/archive/00000496>

Citation for the publisher's version:

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## The effect of static incubation on the yolk sac vasculature of the Japanese quail (*Coturnix c. japonica*)

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Static incubation affects early embryonic development with, notably, a reduction *area vasculosa* expansion and diminished sub-embryonic fluid (SEF) volume, effects produced during a 'critical' period (3-7 days in the chick) (Baggott *et al.*, 2002). Also, as noted by Babiker & Baggott (1992), SEF is produced in bulk only after the appearance of the yolk sac vasculature (YSV), which undergoes extensive proliferation before and during the critical period.

Quantification of such changes in YSV requires estimates of both the quantity of vessels and the degree of branching. In the chick, total vessel length increased linearly up to 160h of incubation, whereas branching was maximal by about 96 h (Vico *et al.*, 1998); so, by the critical period branching is complete yet vessel growth continues. It would seem likely, therefore, that a lack of turning would reduce both measures of YSV proliferation during the critical period.

Eggs were incubated at 37.6°C, 60% R.H., and turned hourly (90°) or left unturned in the same incubator. After 72, 96, 120, 144 and 168 hours of incubation the same area of the *area vasculosa* was removed, stained with 0.05% diaminobenzene in 0.1% hydrogen peroxide, and photographed with an Olympus C-4000 Zoom camera. Images were converted to greyscale and contrast maximised in Adobe Photoshop v 5. The branching of the EV was quantified using the mean box counting fractal dimension (D), computed by Image J with the Fraclac plugin. The quantity of vessels was assessed by a vascular density index (VDI) computed as the number of vessel intersections per mm of test line. A 1mm grid overlaid the image and the number of intersections counted for 5 horizontal lines each of 8 mm in length. Each vessel was counted only once to the fourth level of branching. No distinction made between arterial or venous vessels. For both D and VDI a two-way ANOVA was used with the factors unturned and turned eggs at the 5 periods of incubation; mean comparisons were by the Tukey test (error rate 0.05). Values are expressed as mean±standard error.

Over all periods the mean VDI was smaller in unturned eggs ( $F_{1,110} = 14.81$ ,  $P < 0.001$ ; mean difference  $0.32 \pm 0.08 \text{ mm}^{-1}$ ). However, the period of incubation also affected VDI ( $F_{4,110} = 5.83$ ,  $P < 0.001$ ); there was no significant interaction. Notably, the mean VDI was smaller in unturned eggs at 120 h of incubation compared with turned (and unturned) eggs at both earlier and later time points (Figure 1). Furthermore, unturned eggs had a lower mean D over all periods ( $F_{1,110} = 8.47$ ,  $P = 0.004$ ; mean difference  $0.019 \pm 0.007$ ). The period of incubation also affected the mean D ( $F_{4,110} = 3.80$ ,  $P = 0.006$ ); there was no significant interaction. D was significantly smaller in unturned eggs at 96 and 120h when compared with 144 h of incubation (Figure 2).

Interestingly, the effect of static incubation seems not to be simply due to retardation of YSV proliferation, as VDI was reduced in unturned eggs in the middle of the critical period, only to increase again by 168 h. Also early in the critical period D was 1.70 (as in the chick, Vico *et al.*, 1998), yet then decreased in unturned eggs, although not significantly, and subsequently an increase occurred. Thus during the critical period static incubation specifically affects the structuring of the YSV but whether this is because of, or independent of, retardation of *area vasculosa* expansion is not known.

## References

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Figure 1. The vascular density index, VDI, (mean±SEM, n=6) of the blood vessels of the yolk sac vasculature in turned and unturned quail eggs incubated for different periods. Means sharing the same upper and lower case letters differ at  $P<0.02$ .

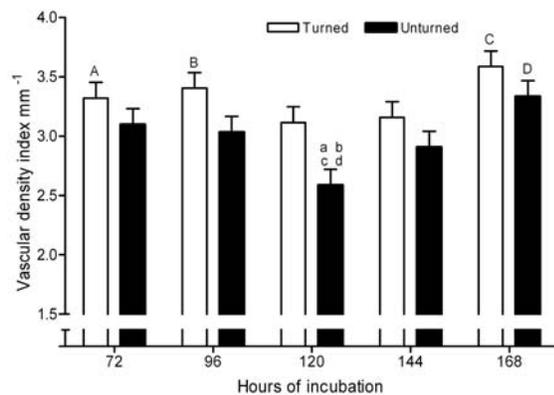


Figure 2. Fractal dimension, D, (mean ±SEM, n=6) of blood vessels of the yolk sac vasculature of turned and unturned quail eggs incubated for different periods. Means sharing upper and lower case letters differ at  $P<0.03$ .

