Can ovarian follicles fossilize?

ARISING FROM X. Zheng *et al. Nature* **495,** 507–511 (2013)

In a recent report Zheng *et al.* describe ovarian follicles in three fossil birds from the Early Cretaceous period of China belonging to *Jeholornis* and two enantiornithine species[1]. Because these were situated in the left half of the body cavity of the fossils, the authors suppose that the right ovary was already reduced in these early birds[1]. Fossilization of ovarian follicles would constitute an extraordinary case of soft tissue preservation, but the morphology of the fossil structures does not agree with the ovulation mode of coelurosaurs. There is a Reply to this Brief Communication Arising by O'Connor, J., Zheng, X. & Zhou, Z. *Nature* **499,** http://dx.doi.org/10.1038/nature12368 (2013).

The Liaoning lagerstätten are renowned for many exceptional examples of soft tissue preservation in tetrapods[2]. However, integument preservation is usually due to fossilization of melanosomes[2,3], and unambiguous evidence for the preservation of less resistible, melanosome-free tissue, such as muscles or internal organs, is scarce (note that the liver, which is sometimes preserved in fossils, contains a high amount of melanosomes). Although fossilized muscle fibres and gastrointestinal tracts of dinosaurs were reported[4], some records, such as that of a supposed dinosaur heart[5], were quickly refuted[6].

In any case, the isolated preservation of easily perishable internal organs without fossilization of more durable soft-tissue structures, such as muscles or integumentary appendices, would be remarkable. In fact, two of the specimens reported by Zheng *et al.*[1] do not show any traces of feathers, and specimen STM29-8 became fossilized in an advanced state of decay, with bones of the pectoral girdle being disarticulated. As can be observed in dissections of decomposed avian carcasses, the gonads are among the first visceral organs to fall victim to decay. Thus, it would be highly unexpected if follicles were the only preserved soft tissue structures. The assumption of Zheng *et al.* that mature follicles could have been preserved owing to fossilization of the "perivitelline layer and other protective layers"[1] is not well founded, because in birds this layer consists of glycoproteins[7], which are unlikely to fossilize.

The presence of up to 12 or 20 equal-sized mature follicles in the specimens reported by Zheng *et al.* would suggest simultaneous ovulation of many follicles, as in crocodiles. However, there exists evidence for paired shelled eggs in compsognathids[8] and oviraptorosaurs[9], and the eggs are arranged in pairs in the nests of oviraptorosaurs and troodontids[10]. This indicates that the avian ovulation mode, that is, the consecutive maturing of follicles, was already present in coelurosaurs, although these still retained two functional ovaries[11]. As a consequence, distinct size differences would be expected among maturing follicles of early Cretaceous birds.

It is also remarkable that the diameter of the largest "follicles", 8.8 mm, is the same in all three specimens reported by Zheng *et al.*, despite the fact that these animals differ greatly in size. We further note that interpretation of similar-sized, spherical structures in the holotype of *Compsognathus* from the Solnhofen limestone as eggs is likewise disputed[12,13].

Although gingko ovules from Liaoning have a similar shape and size[14], we agree with Zheng *et al.* that the morphology of the spherical structures in the bird fossils do not conform with those of 'seeds' (that is, fruit stones). However, in addition to fruit stones there existed other objects in Cretaceous ecosystems that could have been ingested by these birds, such as the fleshy arils of gymnosperms. Fossilization of such organic material in the acidic milieu of the stomach seems more likely than a selective preservation of soft tissue within the body cavity[15].

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Zheng *et al.* reply

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Our explanation that structures preserved in three Early Cretaceous Jehol birds[1] are ovarian follicles is challenged by Mayr & Manegold[2]. We believe that their conclusions are speculative and do not take into account our original arguments. Contrary to Mayr & Manegold[2], unambiguous evidence for the preservation of less resistant tissue, such as muscles or internal organs, are not scarce among Jehol fossils (for example, fish, lampreys)[3] and eggs are sometimes preserved in specimens of the sturgeon *Peipiaosteus* (J.-Y. Zhang, personal communication). Although we cannot explain the vagaries of taphonomy that lead to the preservation of ovarian follicles in these specimens, what is clear is that exceptional preservation of soft tissue is dictated by the unique chemical micro-environment created by the individual decaying tissues, and thus varied degrees of preservation within a single specimen is expected[4]. Exceptional Jehol fossils are a reminder that simply because something is unlikely to preserve does not mean that it will not.

All of the structures interpreted as eggs in *Compsognathus* are not *in situ*[5], making their association more tenuous. However, claims that their small size and large number relative to the eggs preserved in *Sinosauropteryx* refute this interpretation[6] are in fact consistent with their reinterpretation as ovarian follicles[5]. Although these authors doubt the potential for glycoproteins to preserve[1], they have been reported previously in 80-million-year-old mollusc shell[7].

The most plausible alternative interpretation of the circular structures is that they are gut contents, although this alternative is not well supported[2]. First, the anatomical position of the structures is consistent of the position of the ovary and not the ventriculus, which is more ventrodistally located[8]. This is confirmed through comparison with many Jehol birds in which the contents of the ventriculus are preserved. The mass is too caudally located to be the crop, which is cranial to the chest aperture[9]. Second, despite thousands of specimens, no enantiornithine from the Jehol has preserved gut contents; thus, the alternative interpretation conflicts with data that show no indication that enantiornithines were herbivorous (to the contrary, they have robust teeth) or even capable of digesting such foods — no geo-gastroliths, commonly preserved in ornithuromorphs, are preserved in enantiornithines[10]. Gastroliths are absent in all specimens in which follicles are preserved; if these were indeed plant ovules, evidently no grinding mechanism was present to process them. Although Jehol *Gingko* ovules are similar in size, the preserved structures lack ornamentation and other morphological features unique to seeds. Although as paleontologists we imagine that these structures could be plant in origin, paleobotanist E. M. Friis did not consider this to be a possibility[2].

Despite reports that crocodilians ovulate *en masse*, like birds, only one egg can enter the oviduct at a time, thus crocodilians do have a follicular hierarchy. Owing to their much lower metabolic rate, yolk deposition occurs over an extended period, producing only a slight difference in size between mature follicles[11]. The almost, but not exactly equal size of the preserved follicles is a result of the lower metabolic rate of basal birds, which is confirmed by histological studies[12] and is consistent with their intermediate position between crocodilians and extant birds. The similar size of the follicles between the specimens may be due to constraints on the plesiomorphic egg size set by the distally contacting pubes (absent in Neornithes)[13].

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