Validation of Techniques

The efficacy of the technique for examining degraded eggs, described in [1], has been questioned [2]; it has been suggested that sperm on the PVL deteriorate during incubation, even without embryo development. We therefore used fertile turkey (*Meleagris gallopavo*) eggs (n = 48; obtained from Cranberry Foods, North Yorkshire) to test whether this was the case prior to conducting our study. All turkey eggs were laid on the same day, each by a different female from a mature flock, artificially inseminated with a standardised dose of semen 3 days previously. Eggs were collected on the day they were laid, and immediately shaken vigorously to disrupt the PVL in order to arrest embryo development. Inducing embryo mortality at an early stage allowed us to monitor the persistence of sperm on the PVL over a prolonged period of incubation, because it prevented the PVL from becoming increasingly thin and fragile (as it does when the embryo is developing).

The eggs were divided at random into 4 treatment groups of 12 eggs each: Group (1) was not incubated, and the eggs were examined immediately; Group (2) was artificially incubated for 5 days; Group (3) was artificially incubated for 10 days; Group (4) was artificially incubated for 20 days. All eggs were subject to identical conditions during incubation (36.7°C and 60% relative humidity) and all treatments began on the same day as collection. When the incubation treatment was complete, eggs were chilled to 4°C and stored for up to 2 days. Eggs were opened and the contents emptied into a large dish. The perivitelline layer was retrieved, and examined for the presence of sperm as described in [1]. In Groups (3) and (4), 2 and 3 eggs had degraded too much for examination, so 10 and 9 eggs (respectively) were examined.

Sperm were found on the PVL of eggs from each of the 4 treatment groups, including those incubated for 20 days. The number of sperm did not decrease significantly with incubation period (GLM: d.f. = 42, t = 0.95, p = 0.35; Fig. S1). Thus turkey sperm do not deteriorate to the point that they are no longer detectable on the PVL with increasing time in incubation.
References


Fig. S1. The number of sperm counted on 1-cm² PVL from turkey eggs that were (1) not incubated (day = 0; n = 12), (2) incubated for 5 days (n = 12), (3) incubated for 10 days (n = 10) and (4) incubated for 20 days (n = 9). Bold lines indicate the median sperm number for each group; boxes represent the inter-quartile range (IQR); whiskers extend to 1.5 IQR; outlying data points are indicated by open symbols. The number of sperm associated with the PVL did not decrease significantly over the incubation period.