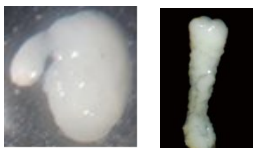
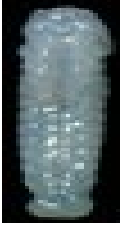
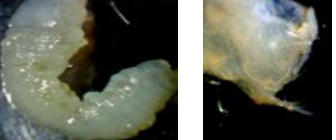
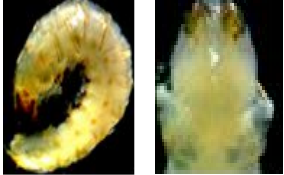


Establishment of Muga Silkworm Embryonic Chart

The embryo isolation protocols *viz.*, Potassium hydroxide (KOH) and hot water methods were slightly modified and utilized. Isolation of early and mid-developmental stages of embryo hot water method was found most suitable. Eggs dipped in hot water at 65 - 78°C for 5 – 10 minutes, depending on the age of the embryo, were glued on egg sheet, dried and dissected under zoom microscope and embryos were isolated. After 120 h of embryonic age, KOH method was employed for isolation of embryos as this method was found suitable in later embryonic stages. The chronological embryonic developmental stages were identified and Muga embryonic chart was established and identified suitable cold sensitive stages for seed preservation.

Muga Eggs: Hours/Days	Morphological Development of Embryo
 20-22h:	Thick germ band developed on the ventral side and it is independent from the egg surface. The Germ band appeared as concave on the inner side and the shape is of an oval plate.
 24 – 26h / (Day-1):	The embryo is in spoon shaped. Embryo has an enlarged head (protocephalon) and tail region (caudal lobe). The cephalic and caudal ends of embryo curved into yolk.
 36-38h (Day-1.5) (Longest embryonic stage):	The embryo occupies 3/4 th of the circumference of the egg. The cephalic/head lobe is further bifurcated and caudal end is pointed. Segmentation can be noticed at this stage.
 48-50h / (Day-2):	Embryo segments are distinguished clearly. A total 18 segments can be distinguished as four segments of cephalic (head) region, three of the thorax and eleven of the abdomen. Neural groove is formed and visible for clear identification. Thoracic appendages appeared as rudiments.
 58-60h (2.5days):	At this stage appendages start appearing in the gnathal region of the head.

 70-72h (3 days):	Embryo length declined gradually. Segmentation initiated in thoracic appendages. In the abdominal region, each somite started producing a pair of appendages in the form of buds from 3rd to 6th segments and on the eleventh segment.
 86-88h (3.5 days):	Thoracic appendages developed in to three segmented legs and can be observed very neatly. In the abdominal region, buds from 3rd to 6th segments and on the eleventh segment appear more clearly
 92-94h (4th day):	Blastokinesis already started in abdominal region of the embryo and is clearly visible and by 4.5 days onwards embryo assumed 'S' shape.
 120 h (5th day) :	Blastokinesis is completed and embryo moved to dorsal side. This process completed at the beginning of 5th day.
 144 h (6th day) :	Segmentation begun at labrum and labium. Antennae and caudal horn started appearing and is clearly visible at 156h. The spiracles started appearing on the lateral sides of the embryo.
 168 h (7th day) :	Setae started forming on the surface of the embryo and spiracles are very clearly distinguishable. Gnathal appendages sclerotized and started appearing brown in color. Claws are visible in thoracic appendages. At the distal end of abdominal appendages sclerotisation started.
 192h (8th day) :	Head capsule, mouth appendages and thoracic segments are completely sclerotized and appeared thick blackish brown in color (Similar to pin head stage of Mulberry silkworm (<i>Bombyx mori</i>)). Ocelli are very clearly seen at this stage. Setae well developed on body.

 216h (9th day) :	Body completely sclerotized and setae are sharp on the body and completely covered. Embryo color shows a combination of mixed yellow and brown. Embryo is ready to hatch at this stage .
 240h (10th day Morning) :	Larva comes out by rupturing the anterior part of the egg shell in semi dark condition.

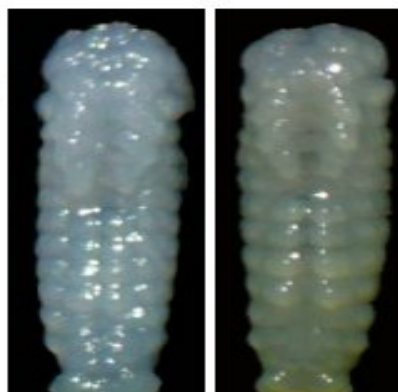


Fig 8: Cold se
nsitive ages suitable for Muga silkworm seed preservation