EMBRYO ISOLATION AND EGG PRESERVATION TECHNOLOGY OF ERI SILKWORM *SAMIA RICINI* (DONOVAN) (LEPIDOPTERA: SATURNIIDAE)

B. N. Sarkar*, M. C. Sarmah*, P. Dutta and K. Dutta**

* Central Muga Eri Research & Training Institute, Central Silk Board, Jorhat-785700, Assam, INDIA. E-mail: bnsarkarcsb@yahoo.com
** Department of Zoology, Gauhati University, Gauhati – 781014, Assam, INDIA.

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ABSTRACT: Embryo isolation and egg preservation is one of the important aspects for synchronizing the eri silkworm Samia ricini Donovan rearing activities. The experiment of embryo isolation and preservation of eri eggs revealed that the 36-40 hrs. old age group eggs can be preserved maximum days without any adverse affect. This group of eggs hatched after 25 days of oviposition and the bioassay of cocoon weight (3.2gm), shell weight (0.42gm) and ERR (68%) is significant. From the study, it is indicated that multivoltine eri eggs can be preserved in the particular embryonic stage and ages.

KEY WORDS: Embryo isolation, different stages, preservation of eri silkworm egg.

The eri silkmoth *Samia ricini* (Donovan) belongs to the family Saturnnidae is the only domesticated species under genus *Samia*. Eri silkworm is multivoltine in nature and 4-5 brood can be reared throughout the year providing the primary food plants are available and favourable climatic conditions (Sarkar, 1988).

The Brahmaputra valley of Assam, India and its adjoining area is believed to be the original home and traditional of cultivated eri silkworm. *Samia ricini* (Lepidoptera: Saturniidae) is also reared in Sri lanka, Thailand, Egypt, Lebanon, Korea etc.

Egg is considered as the key factor of sericulture industry. Only quality eggs can ensure a good harvest and healthy crop. Although eri silkworm rearing is conducted through out the year, the seed production is not suitable in all the seasons (Sarkar et al., 2010). On the other hand, the embryonic development stage in silkworm is very susceptible to environmental conditions i.e. temperature, humidity etc. (Choudhury, 2005) which is greatly influenced by quality seed cocoons. Embryological study in silkworm is one of the important aspects as different embryonic stages response differently to different temperature and humidity, which is key information required for the preparation of silkworm eggs preservation schedule. Eri silkworm eggs are cleidoic system where no exchange of materials through chorion except gases occurs. The surface and structural elements of the egg chorion of eri moth, viz., the micropylar rosette surrounding the micropyle, micropylar canals, shell imprints, aeropyles and the regional differentiation at the different poles (Kumar, V. et al., 2007). The protection against environmental hazards during embryogenesis is one of the functions of the eggshell. The embryonic study of eri silkworm was carried out by Narasimhana (1964), Rahman & Mahanta (1985). But the technique adopted by most of the authors are on microtome section studies. Takami & Kitazawa (1960), made the numerical nomenclature on different embryonic stages in *B. mori* on the basis of external morphology.

Keeping all these in view, the present study was under taken to develop long term egg preservation technique with embryological study for availability of

sufficient quantity of seed as synchronized scheduled time or overcome the unfavorable season and supply as per demand.

MATERIAL AND METHOD

Eri silkworm eggs were collected from Eri Germ Plasm Bank of Central Muga Eri Research and Training Institute, Central Silk Board, Lahdoigarh, Jorhat, Assam, India. To find out the most suitable embryonic stage for long term preservation, the standard technique developed by Reddy et al. (2003), in mulberry silkworm applied with modification for eri silkworm eggs due to somewhat different to the mulberry eggs i.e. shape, size and chorionic layer covered with thick gummy substances to make uniform cluster (Fig. 1).

Isolation of Eri Embryo:

Different hour ages of eri silkworm eggs were taken as experiment materials for eri embryo isolation. When the pairing of male and female eri moth was 7am - 10 am, the zero hour considered 6 - 8 pm on the same day and next day morning 8 am considered as 12 hrs. egg. When the pairing time was 7am - 10 am, next day evening 8 pm was considered as 24 hrs.egg. After collection of different hours old eggs from *Kharika* (ovipositional device) the clustered eggs were treated 3-5% KOH solution (3-5 gm/100 ml of water) and treated in water (60-70^{o}C) for 2-5 minutes only on the basis of ages of embryo. After boiling, cool the sample eggs in running water and following washed in the distilled water with a tea sieve for few minutes. The treated eggs now preserved in the distilled water in a transparent glass petridis under the dissection microscope Kyowa-Gentner (OPZ-PLS) Incident 6v1.2A for isolation of embryo. For the dissection and embryo isolation the treated eggs were fixed with a surgery blade and the chorion of posterior region sliced by the sharp corner of new blade. The solid embryo of eri eggs easily come out by removing chorion with forcing of surgery blade (no.10).

Best result were found when the treated eggs materials kept in the normal freeze for 12 hours. After normal freezing the embryo become smaller, solid and chorion become transparent, which is easy to dissect and solid embryo of eri eggs easily come out after removing the chorion of the treated eggs. After isolation of different stages embryo were preserved in the 70% alcohol in the small glass vial for permanent slide preparation. The permanent slide were prepared whole mount preparation methods by Reddy et al. (2003), and taken photograph (Leica DM 3000 fluorescent Microscope with camera and accessories).

Preservation of Egg:

The eri silkworm egg produced in the favourable temperature and humidity condition to conduct the experiment. For preservation of eri eggs, different age group of eri eggs viz.-12 hrs. old egg, 24 hrs. old egg, 36 hrs. old egg and 48 hrs. old eri eggs were preserved in the 5^{o}C temperature and 75-80% relative humidity in BOD. There are eight replication of eri silkworm egg of total 40 disease free layings (dfls) were preserved in each group and each replication 5 dfls were kept for preservation for all of four age group of eri eggs. The eri silkworm eggs preserved in descending order of temperature viz. 20^{o}C (2hrs.) >15^{o}C (2hrs.) >10^{o}C(2hrs.) >5^{o}C(constant) to avoid thermal shock or injury. Preservation temperature was fixed 5^{o}C recommended for silkworm egg preservation technique. Released the eggs after 5 days interval from preserved temperature in the exposing at the ascending order viz.5^{o}C >10^{o}C (2hrs.) >15^{o}C (2hrs.) >20^{o}C (2hrs.) to avoid cold injury. The preserved eggs generally release @ 5 dfls after 5

days interval in the cool hour of day after proper incubation in the temperature of 25°C. A lot of eri eggs were kept in control without preservation for comparison. After preservation the maximum days prolonged group was reared and data were compared with the control group. Evaluated the total days of preservation, incubation period, hatched after total days of oviposition, hatching %, unhatched %, larval weight, cocoon weight, shell weight, ERR% was considered.

RESULT AND ANALYSIS

Isolation of Embryo:

Embryo isolated from the stage of 15 onwards and it was found that the isolation of late age embryo were easy than young age embryo. Diiferent stages of eri embryo development are described below (Fig.1).

Stage-15: In 15 stages the embryo developed into segmentation with enough length stage and the amniotic fold covering the embryo was clear and serosa was completely covered with the yolk. The embryo was slender with a well-defined head and caudal region. The head has a clear cut depression in the middle. Eighteen mesoderm segments are clearly visible.

Stage-17: Rudiments of appendages appear in thoracic region and cephalic region formed by the beginning of stomodaeum.

Stage-21: The process of blastokinesis begins after 4.5-5 days after oviposition. Embryo starts to move around and the embryo assumes S shape. Blastokinesis first start in the abdominal region and extend toward heads. Posterior abdominal segments are first turned vertically so that the abdominal region as a whole forms a straight line. The abdominal region then turns towards anterior side and reaches the level of prothorax. The anterior and posterior ectodermal invaginations extends to form the fore gut and hind gut respectively.

Stage-22: After 5 days of oviposition inversion is completed and the embryo move towards dorsal side from ventral side. The yolk is also taken in when the dorsal side of embryo closes. Yolk mass inside the eggs serves as a source of nutrients for the developing embryo and also help in holding the embryo on its surface as a necessary foundation.

Stage-23: After 6 days of oviposition lateral walls complete and tips of labrum and labium become segmented. Thoracic legs become segmented with claws at distal end. Rudiments of the setae develop on the body surface.

Stage-24: At about 6.5 days of oviposition the entire body of embryo covered by strong setae and embryonic moult occurs in this stage. The caudal horns also occur in this stage.

Stage-25: At 7 days after oviposition mandible become selerotised and pigmented at distal ends. Larval eyes (i.e. ocelli) appear as six brown spot on either side of head. The spiracles are clearly visible on the sides of the body.

Stage-27: Head capsule and mouth appendages are sclerotised after 8thday of oviposition. The amnion and serosa disappear by fragmentation. Embryo ingests the embryonic membranes and sensitive for adverse environmental condition.

Stage-28: On the eight day after oviposition embryo become scierotised. In this stage embryo was very sensitive to high temperature and low humidity.

Stage-29: On the nine day after oviposition entire body of embryo become scierotised (pigmented stage). Although the chorion was thicker than mulberry silkworm embryo, the blakish pigmented embryo was visible in this stage. The embryo was very sensitive to high temperature and low humidity.

Stage-30 (Newly born eri larvae): Fully developed eri larva come out from the egg cell on the10 days of oviposition rupturing the anterior part of egg shell by

the mandibles and swallowing the portion of the chorion in the early morning of exposure of light. Newly born larvae are generally blackish or brownish in colour. Generally healthy larvae are blackish in colour and brownish colour larvae are somewhat weak. Head portion is shining black with elongated spot and larval body is yellowish with blackish tubercle.

Egg Preservation:

The egg preservation of different age groups showed following result-

12-14 hrs old egg: The experiment of eri silkworm egg preservation revealed that 12-14 hrs.old eggs of eri silkworm were tolerate the preservation only 4 days and where the hatching was 4% and the rearing performance is also not significant.

24-26 hrs old egg: Preservation of 24-26 hrs. old eggs of eri silkworm was tolerating the preservation only 7 days. The hatching was only 40% and ERR% of the rearing was 34%, which indicated that 24 hrs. old eri eggs are also not suitable for long term preservation.

36-40 hrs old egg: When preserved the 36-40 hrs. old eggs of eri silkworm the eggs prolonged up to 25 days and the hatching were found 72%. Maximum gain over control of preservation days is 16 days. The rearing performance was also higher than of all of others age group of eggs of eri silkworm. For the long term preservation of eri silkworm eggs 36-40 hrs. old age are responding for arrest the growth of embryonic development and indicate suitable to preservation of eri eggs for long duration.

48-52 hrs old egg: Eri silkworm egg of 48-52 hrs. old age, maximum only 9 days preserved and hatched after 16 days after oviposition. The gain over control of preservation is only 6 days and hatching was only 13%. The rearing performance indicated that cocoon weight (2.7gm.) and ERR(25%) and it is inferior than to the control group.

DISCUSSION

Different hour old ages of embryo of eri silkworm eggs were isolated following standard methods of silkworm embryo isolation. Different stages of embryo (Fig: 1) found that shape and size of embryo are changing in their growing stages as mentioned above. Also, the egg preservation of different age group of multivoltine eri eggs and result showed that the 36-40 hrs. old age group eggs can be preserved for long time without any adverse affect in embryonic development and rearing performance. This group of eggs hatched after total 25 days of oviposition and gain over control is 16 days. The bioassay of preserved 36-40 hrs. old age group eggs were conducted and the result revealed that the cocoon weight (3.2 gm), shell weight (0.42gm) and ERR(68%) is almost similar to the control group of eri eggs.

Yaginuma et al. (1990), reported that the young age multivoltine silkworm eggs can tolerate cold temperature but not advance embryos since sorbitol appears only during former stage but not in later. Furusawa et al. (1987), stated that the progressive conversion of multivoltine silkworm eggs of sorbitol to glycogen takes place after 40 days of preservation. The same observation is also observed during the preservation of multivoltine eri silkworm eggs. Hence, multivoltine eggs preserved at a temperature of 5 ^{o}C for 30 days do not any adverse affect on the cocoon weight, shell weight and raw silk yield. The resistant of embryo to cold storage and its sensitivity to artificial induction of hatching varies according to the developmental stages and hence determination of development stages of embryo is important by external morphology (Yokoama,

1973). Therefore the identification and characterization of different embryonic stages on morphological characters have its importance as each embryonic stage has got its own tolerance limit in each temperature and humidity condition.

The study of the eri embryo isolation and eggs preservation clearly indicate that 36-40 hrs. old age group and stage (15) of eri eggs can be preserved for longtime and it will help to synchronize the crop schedule. Due to non availability of a suitable technology for the preservation schedule of eggs in eri culture, rearing scheduled cannot be planned as per the availability of leaves and the favorable season, resulting in the poor cocoon harvest. From the study it is indicated that multivoltine eri eggs can be preserved in the suitable embryonic developmental stages for prolongation of hatching and harvesting good crops.

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Table 1. Preservation of Eri eggs (sample size 40 dfls).

Age of eggs (hrs)	Total preservation (days)	Incubation Period (days)	Hatching after total days of Oviposition (days)	Gain Over Control (days)	Hatching (%)	Larval Period (days)	Cocoon Weight (gm)	Shell Weight (gm)	ERR (%)
12 hrs	4	7	11	2	4	21	2.8	0.35	47
24 hrs	7	8	15	6	40	21	3.1	0.41	34
36hrs	17	8	25	16	72	20	3.2	0.42	68
48 hrs	9	7	16	7	13	21	2.7	0.36	25
Control 12 hr.	0	9	10	-	75	20	3.3	0.44	70

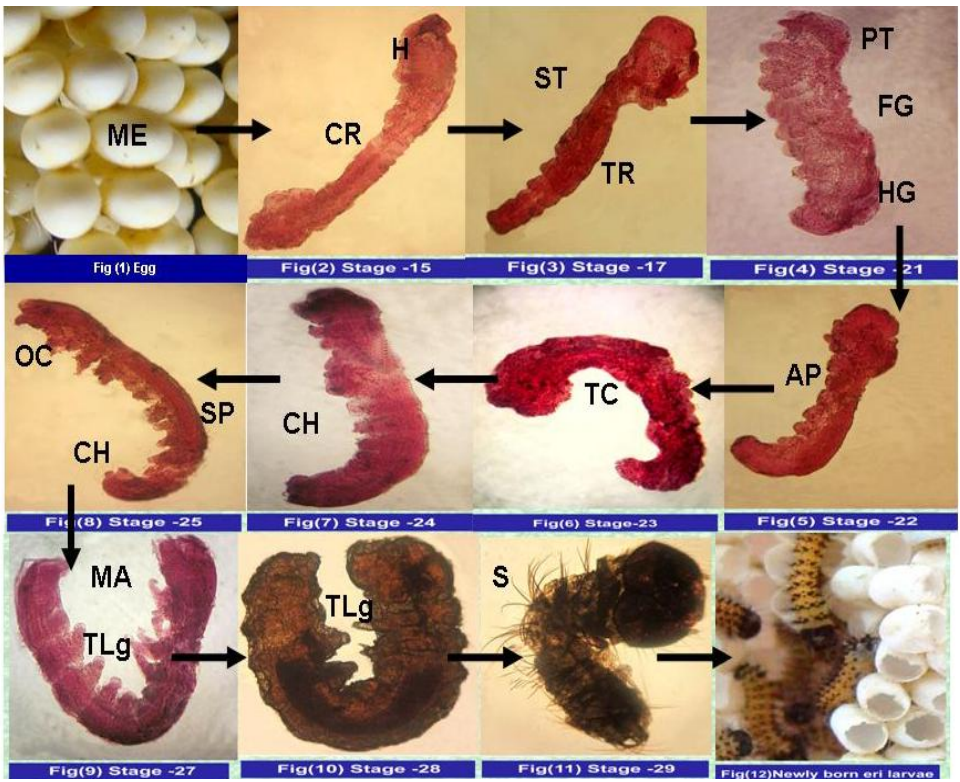

Figure 1. Different stages of embryos after isolation. [ME=Mature Eri eggs, H= Head region, CR= Cephelic region, ST= Stomodaeum, TR=Thoraxic region, PT=Prothorax , FG=Fore gut, HG=Hind gut, AP=Appendages, TC=Thoracic claws, CH= Caudal horns, OC=Ocelli, SP=Spiracles, MA=Mouth appendages, TLg=Thoraxic legs, S=Setaes.]