Data Article

Data of sperm-entry inability in *Drosophila melanogaster* ovarian follicles that are depleted of s36 chorionic protein

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**Abstract**

This paper presents data associated with the research article entitled "Targeted downregulation of s36 protein unearths its cardinal role in chorion biogenesis and architecture during *Drosophila melanogaster* oogenesis" [1]. *Drosophila* chorion is produced by epithelial follicle cells and one of its functional serving role is egg fertilization through the micropyle, a specialized narrow channel at the anterior tip of the egg [2]. Sperm entry during fertilization is necessary for the egg to complete meiosis [3]. *D. melanogaster* flies being characterized by severe downregulation of the s36 chorionic protein, specifically in the follicle-cell compartment of their ovary, appear with impaired fly fertility (Velentzas et al., 2016) [1]. In an effort to further investigate whether the observed infertility in the s36-targeted flies derives from a fertilization failure, such as the inability of sperm to pass through egg’s micropyle, we mated females carrying s36-depleted ovaries with males expressing the GFP protein either in their sperm tails, or in both their sperm tails and sperm heads.

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**Specification Table**

| Subject area                        | Biology                        |
|-------------------------------------|--------------------------------|
| More specific subject area          | Cell and Developmental Biology |
| Type of data                        | Confocal Laser Scanning micrographs |
| How data were acquired              | Using a Nikon Eclipse C1 Confocal Laser Scanning Microscope (CLSM) |
| Data format                         | Analyzed data                  |
| Experimental factors                | Female virgin control and s36-targeted flies were mated with dj-GFP or protamineB-eGFP; dj-GFP males. The deposited eggs were collected every one hour and observed under a Nikon CLSM |
| Experimental features               | Comparison of successful fertilization levels between laid s36-depleted ovarian follicles and control ones |
| Data source location                |                                  |
| Data accessibility                  | All data are included in this article |

**Value of data**

- Insemination and not sperm entry into mature follicles seems responsible for the activation of ovulation process in *D. melanogaster*: new prospects for control of oogenesis by sperm microenvironment.
- Flies carrying s36-depleted ovaries may serve as a primary model system for deciphering the sperm-regulated ovulation and egg-deposition rhythms in *D. melanogaster*, through the use of spermatozoa with various genetic backgrounds.
- Imaging and quantification of *D. melanogaster* fertilization via employment of transgenic -fluorescent- spermatozoa technology most likely provide a useful and valuable platform for the assessment of, other than s36, major chorionic-components’ contribution to follicles’ competence for efficient fecundity.

1. **Data**

   In order to examine *Drosophila melanogaster* sperm’s ability to penetrate ovarian egg’s micropyle [2] and enter into oocyte’s cytoplasm of the s36-downregulated follicles, we mated s36-targeted virgin female flies with males expressing either the don juan-GFP fusion protein (dj-GFP), or both the dj-GFP and Mst35Bb/ProtamineB-eGFP proteins (Fig. 1A and B). The *Drosophila* don juan (dj) protein is expressed along the axoneme of each sperm tail [3–4], while protamineB is specifically localized in sperm heads [5]. To validate sperm’s GFP-mediated fluorescence in the transgenic male flies, their testes expressing either the dj-GFP (Fig. 1A) or both the dj-GFP and protamineB-eGFP proteins (Fig. 1B) were visualized under a CLSM, clearly revealing bright green staining patterns for both spermatozoa populations examined.

   More than half in number of the freshly-laid eggs (n=90) obtained from control (c355-GAL4/+) female flies after they have been crossed to males expressing dj-GFP (Fig. 1C and G) proved to be successfully fertilized, with GFP-tagged sperm being readily detected in their cytoplasm. Similarly, a 67% mean value of laid eggs (n=105), derived from control female flies mated with protamineB-eGFP; dj-GFP transgene-carrying males, were also presented with GFP-tagged sperm (see, its coiled shape within the anterior region of the herein shown representative follicle) inside each fertilized egg’s cytoplasm (Fig. 1D and G). In contrast, GFP-tagged sperm could not be detected inside the cytoplasm of the freshly-laid s36-depleted eggs produced by female flies that have been inseminated either by dj-GFP (n=110; Fig. 1E and G) or by dj-GFP and protamineB-eGFP transgene-containing males.
Fig. 1. Fertilization inability of s36-depleted fly follicles results from sperm-entry failure. CLSM images of spermatozoa inside testes, expressing (A) the don juan (tail-specific) or (B) both the don juan and protamineB (head-specific) GFP-conjugated protein markers. CLSM images of laid fertilized eggs, as demonstrated by the GFP-tagged sperm inside each cytoplasm, after crossing control (c355-GAL4/+ ) female flies to (C) dj-GFP or (D) protamineB-eGFP; dj-GFP transgene-carrying males. Representative CLSM images of laid follicles, with no GFP-tagged sperm detected in any respective cytoplasm, having been derived from s36-targeted (c355 > s36_RNAi) female flies after their mating with (E) dj-GFP or (F) protamineB-eGFP; dj-GFP transgene-containing males. (G) Graphic presentation of the percentage (%) of fertilized eggs, as indicated by the entry of fluorescent sperms through ovarian-follicles’ respective micropyles, for each one of the genetic backgrounds described above. Arrowheads point spermatozoa and arrows indicate dorsal appendages. Scale bars: 50 μm.
Interestingly, insemination (introduction of semen into the female animal), and not sperm penetration into the mature follicle, seems to represent a sufficient factor for triggering the ovulation process in *D. melanogaster*, since no statistically significant difference in the egg-deposition capacity could be observed between control and s36-targeted flies.

2. Experimental design, materials and methods

2.1. *Drosophila melanogaster* strain stocks and maintenance

For this study, the following *D. melanogaster* transgenic fly strains were used: P[w+mW.hs]=GawB)c355, w[1118] (BL: 3750), w[*]; P[w+mC]=protamineB-eGFP)2/CyO; P[w+mC]=dj-GFP.S)3/TM3, Sb[1] (BL: 58406) and w[*]; P[w+mC]=dj-GFP.S)AS1/CyO (BL: 5417), all obtained from Bloomington *Drosophila* Stock Center (Indiana, USA), and UAS-s36_RNAi (Transformant ID: 14824), provided by Vienna *Drosophila* RNAi Center (Vienna, Austria). Fly stocks maintenance was performed as previously described [1].

2.2. *Drosophila melanogaster* mating, egg collection and CLSM imaging

Control (c355-GAL4/+ ) and s36-depleted (c355 > s36_RNAi) virgin female flies (3–5 days) were mated overnight with either dj-GFP.S or protamineB-eGFP; dj-GFP.S male flies. Female flies were left to lay their eggs in standard apple-juice agar plates and the obtained eggs were being collected every one hour and immediately observed under a Nikon confocal laser scanning microscope (CLSM), model Digital Eclipse C1 (Nikon; Tokyo, Japan).

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Transparency document. Supporting information

Transparency data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.dib.2017.03.052.

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