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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

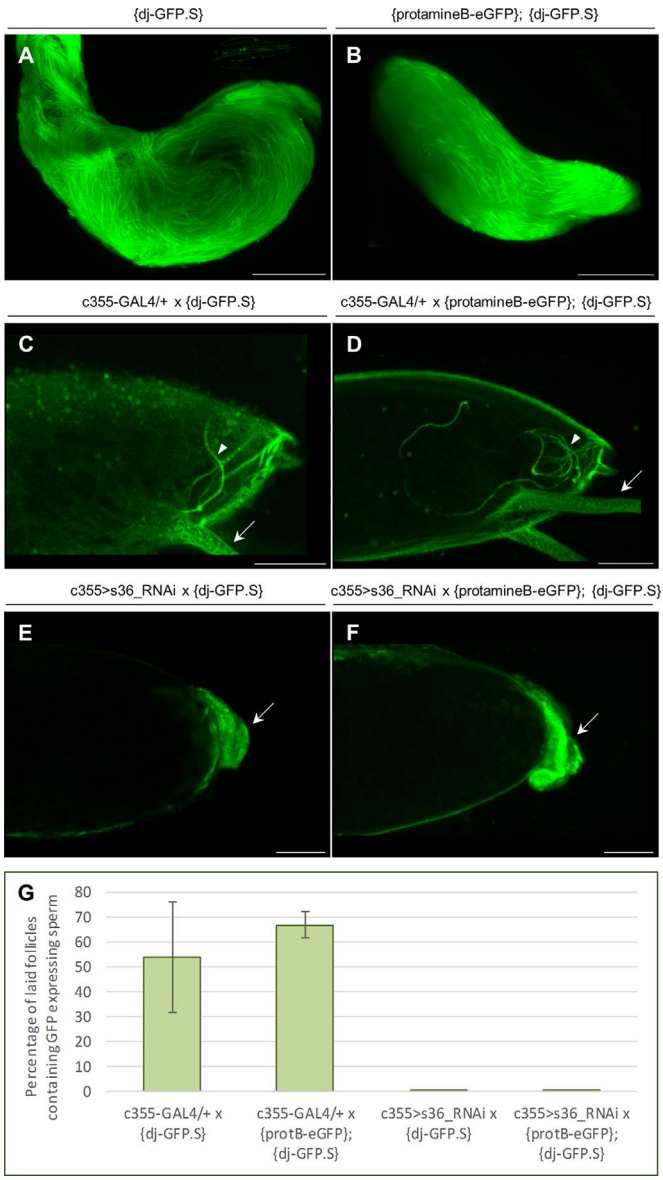

Fig. 1. Fertilization inability of s36-depleted fly follicles results from sperm-entry failure. CLSM images of spermatozoa inside testes, expressing (A) the don juan (tail-specific) or (B) both the don juan and protamineB (head-specific) GFP-conjugated protein markers. CLSM images of laid fertilized 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.

($n=120$; Fig. 1F and G). 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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The authors wish to thank Bloomington Stock Center (Indiana, USA) and Vienna *Drosophila* RNAi Center (VDRC) (Vienna, Austria) for fly stocks. Dimitrios J. Stravopodis would like to devote the present article to the memory of his precious mother who so suddenly passed away in October 2015.

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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