Maternal age does not affect embryonic viability or fertilization success in *Drosophila melanogaster*

Dawn Comstock and Hallee Adamsheck
Gustavus Adolphus College: St. Peter, MN

Introduction

Maternal aging is an important factor in reproductive success because it can affect oogenesis, offspring viability, and developmental outcomes. In humans it has been shown that infertility increases with maternal age, while embryonic viability decreases with maternal age[1]. Previous research has also shown the detrimental effect of maternal age on reproductive success in *Drosophila* as old females had lower mating success and fecundity[2]. This study is intended to further examine the effect of female age on components of reproductive success in *Drosophila melanogaster.* Possible factors contributing to a difference in embryonic viability include fertilization success and embryo cytoskeleton structure. In *Drosophila* embryos the cytoskeleton has been shown to play an important role in migration of nuclei towards the cortex and cellularization which divides the syncytial cytoplasm into mononucleated cells[3].

Our objective was to analyze the effect of maternal aging on reproductive success by examining embryonic viability, and if a difference exists, whether this can be attributed to fertilization success or differences in cytoskeleton structure in *Drosophila melanogaster* embryos.

We hypothesized that increased maternal age is correlated with decreased embryonic viability, shown by fewer successful hatchings, that old females will have lower fertilization success indicated by a higher incidence of ovulated eggs not containing sperm and there will be a difference in the amount and location of actin filaments present in the embryos of old and young females.

Methods

Establishment of *Drosophila* Stocks

Oregon-R, wild type, stock of *D. melanogaster* were mated. From these matings virgin females were collected and aged to either 32 days (“Old” Females) or 3 days (“Young” Females). Males producing GFP-labeled sperm (protamine-GFP and dj-GFP) were obtained from stock maintained by the Bloch Qazi lab.

Embryonic Viability

Old and young females were inseminated by mass matings with collected males, this was repeated for young Females. Embryos were collected on grape plates. After 24 hours 25 embryos from each treatment were transferred to new grape plates. These were observed after 36 hours to determine number hatched (larva) and unhatched (intact embryos).

Fertilization Success

Embryos were fixed using the fast-fix formaldehyde protocol[4]. Staining was conducted using Phallodin to stain for actin filaments and DAPI to stain for nuclear components.

Methods

Visualization

Embryos were visualized at 10x magnification using a Zeiss LSM 700 laser (scanning) confocal microscope on widefield view. Images were collected using Zen 2009 software.

Results

Embryonic Viability

Of the 25 embryos collected and observed after 36 hours:

- In both old and young females fertilized with protamine-GFP males, 13 embryos hatched. The percentage of hatched embryos was 48% in both groups.
- In dj-GFP fertilized old females resulted in 5 hatched embryos, whereas the young females had 8 hatched. X^2_1=1.6542; 0.1<p<0.25.

Fertilization Success

- In total 12 embryos and oocytes from all treatment groups were successfully mounted onto the slides.

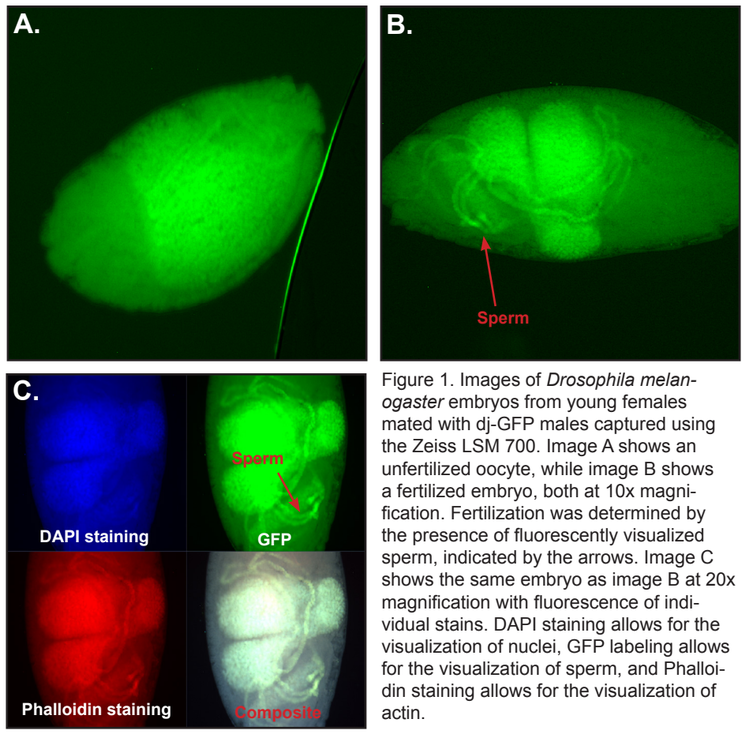

Figure 1. Images of *Drosophila melanogaster* embryos from young females mated with dj-GFP males captured using the Zeiss LSM 700. Image A shows an unfertilized oocyte, while image B shows a fertilized embryo, both at 10x magnification. Fertilization was determined by the presence of fluorescently visualized sperm, indicated by the arrows. Image C shows the same embryo as image B at 20x magnification with fluorescence of individual stains. DAPI staining allows for the visualization of nuclei, GFP labeling allows for the visualization of sperm, and Phalloidin staining allows for the visualization of actin.

Discussion

There was no observed effect of aging on embryonic viability. There was no statistical difference between hatching success of old versus young females in protamine-GFP fertilized. The difference in successful hatchings in dj-GFP fertilized females was different numerically, however, it did not show statistical difference.

Due to the small number of embryos retained through staining and fixation, we did not have sufficient data from the represented treatment groups to allow for statistical analysis. There was insufficient data to drawn any conclusions regarding old versus young fertilization success. We did however accomplish the ability to detect fertilization through fluorescent microscopy. Presence of sperm was detectable in fertilized embryos using widefield view.

Qualitatively we noticed differences between the general embryo shape of fertilized and unfertilized embryos, as unfertilized embryos slightly collapsed and did not retain the characteristic elipsoid. We were unable to assess if these differences were due to actin, because of saturation of the fluorescence and background in widefield view.

Future Directions

- The difference between viability of dj-GFP versus protamine-GFP fertilized embryos would be an interesting follow-up study. Because of the difference in hatching success between these two groups, one could study the effect of different male lines on reproduction.
- Refine fixation and staining techniques to maximize the number of embryos retained.
- Repeat the staining protocols and image embryos using confocal microscopy techniques.

Figure 2. Problem solving to improve mesh baskets for embryo washing and dechorination. During this portion of the protocol we lost many embryos and hope that smaller mesh baskets will improve embryo retention.

Acknowledgements

A special thanks to Dr. Margaret Bloch Qazi for her assistance and guidance in all drosophila related matters and for providing the fly stocks. Dr. Karla Marz for supplying fluorescent dyes. Maureen Carlson for her insight and advice regarding materials. Dr. Jeff Dahlseid for teaching us how to use the Zeiss LSM 700 microscope. The Gustavus Biology Department for funding.

Literature Cited

1. **O’Keane, J.** Aging and Reproduction (1997). *ACOG Clinical Review* **2**, 9.
2. **Somashekar, K., Krishna, M. S., Hegde, S. N. and Jayaramu., S. C.** (2011). Effects of age on female reproductive success in Drosophila bipectinata. *Journal of Insect Science* **11**, 1-16.
3. **Foe, V. E., Field, C. M. and Odell, G. M.** (2000). Microtubules and mitotic cycle phase modulate spatiotemporal distributions of F-actin and myosin II in Drosophila syncytial blastoderm embryos. *Development* **127**, 1767-87.
4. Drosophila Protocals. Sullivan et al., 2000, Cold Spring Harbor Laboratory Press.