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DETERMINATION OF DRY MASS AND AREA OF ATTACHED AND DETACHED CYTOPLASMIC DROPLETS OF *AMBYSTOMA TEXANUM* SPERMATOZOA USING QUANTITATIVE INTERFERENCE MICROSCOPY

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ABSTRACT

There were no differences in dry mass and area of attached and detached cytoplasmic droplets of *Ambystoma texanum* spermatozoa determined using quantitative interference microscopy. The combined means for dry mass and areas of 60 droplets were found to be 24.6 ± 2.6 pg and $2.4 \pm 0.1 \times 10^{-7}cm^2$. The correlation coefficient for dry mass and area is $r = 0.86$.

Wortham et al. (1977) have reviewed literature on cytoplasmic droplets in different salamander species. Russell et al. (1981) recently described their observations for spermatophores and spermatozoa of the salamander *Ambystoma texanum* following light and electron microscopy. In vasa deferentia cytoplasmic droplets were attached to spermatozoan necks and as free droplets in the spermatophore caps where they were internally segregated by a membrane like structure. The free and attached droplets were cytologically alike in the following ways: both types of droplets showed weak reactions with periodic acid-Schiff, Alcian blue of pH 2.5, ninhydrin-Schiff, and osmium tetroxide; in paraffin sections, both types stained orthochromatically with Toluidine blue 0; dark granules were seen in both types with phosphotungstic acid hematoxylin and Heidenhain's iron hematoxylin; and internally, both types showed many membranous cisternae and sacs in addition to two types of dense bodies.

Because spermatozoan cytoplasmic droplets found in many salamander species contained mitochondria, cytoplasmic RNA, and a highly complex membrane system

(Murphy et al. 1973, Wortham et al. 1977) these authors inferred that droplets were functional and played a role in the maturation of spermatozoa or were of significance to spermatozoan motility of fertilizing capacity. Wilder (1913) suggested that cytoplasmic droplets may be an adaptation for prolonged storage of sperm in the female spermatheca or that they resisted dissication of spermatozoa of terrestrial species.

We used quantitative interference microscopy to test the hypothesis that the dry mass and area of attached and detached cytoplasmic droplets in the same environment (fluid from vas deferens) are not statistically different.

Vasa deferentia were excised from one *Ambystoma texanum* collected in Jackson, Co., Illinois and seminal fluid was collected from the distal portion onto glass slides. Coverslips were placed over the samples and ringed with clear fingernail polish. Preparations were viewed with a Letiz transmitted light (Mach Zehnder) interference microscope (50x A 0.85/∞/0.17 objectives, 16x Periplan GF oculars, field dia. 0.3mm. 800x magnification). Optical path differences for 30 attached and 30 detached droplets were recorded. All 60 cytoplasmic droplets were photographed with an Orthomat camera and planimetry of projected and traced images was used to determine area of the cytoplasmic droplets (Kulfinski and Pappelis 1971). Planimetered areas were corrected for the magnification factor and these values and optical path differences measurements were inserted into the formula of Beneke (1966) to determine toatl dry mass. Comparisons were made using Analysis of Variance and Duncan's Multiple range for means separation ($p = 0.05$). Standard error of the means were calculated for total area and total dry mass values.

Total dry mass and total area means for attached cytoplasmic droplets were 24.1 ± 2.6 pg and $2.5 \pm 0.3 \times 10^{-7}cm^2$. For detached cytoplasmic droplets, these means were 25.0 ± 4.6 pg and $2.3 \pm 0.3 \times 10^{-7}cm^2$. Since there were no statistical differences between total area and dry mass values for attached and detached cytoplasmic droplets, these values were combined and resulted in means of 24.6 ± 2.6 pg and $2.4 \pm 0.1 \times 10^{-7}cm^2$. A correlation coefficient between area and dry mass was calculated to be $r = 0.86$. A unit dry mass (total dry mass/total area) of 10.2×10^{-5} g/cm^2 was calculated from the combined values.

We accept the null hypothesis that the dry mass and area means of attached and detached cytoplasmic droplets in the fluid from vas deferens are not statistically different and extend the findings of Russell et al (1981) on the similarities of these two types of droplets. More important to us is the demonstration that quantitative interference microscopy may be useful to researchers seeking to test the null hypothesis that these highly organized structures are functionless cytoplasmic remnants and play no role in motility and storage. Similarly, the method may prove helpful to those who will study the origin of droplets, changes in the droplets during maturation of spermatozoa, and changes in droplets that may occur in senescence of spermatozoa.

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