Evaluation of canine sperm morphology using two techniques for sperm separation
Rachel Hegedus, Michelle Kutzler
Department of Animal and Rangeland Sciences, Oregon State University, Corvallis, OR

Introduction
Veterinarians often encounter subfertile male dogs whose poor quality semen is intended for artificial insemination. Several methods for the elimination of abnormal sperm are available. Density gradient centrifugation (DGCM) and the swim up method (SUM) have both been shown to be effective in separating sperm but their efficacy in recovery of morphologically normal canine sperm has not been compared. The objective of this study was to compare sperm morphology following these two methods of sperm separation. With the success and widespread use of commercially-available DGCM for horses, we hypothesized that this method would yield a higher percentage of morphologically normal sperm than the SUM.

Methods
Semen was manually collected from three dogs who had sired a litter within a year from semen collection. Samples were divided into three aliquots of equal volume. Sperm morphology was assessed immediately prior to sperm separation using an eosin-nigrosin stain and by counting 200 sperm under oil immersion. For the DGCM, Equipure™ (Nidacon International, Mölndal, Sweden) was overlaid with the semen sample and centrifuged for 30 min at 100 x g as previously described for horses. For the SUM, the semen sample was centrifuged twice for 15 min at 215 x g in Ham's F-10 and then incubated at 37°C in 5% CO2 at a 45° angle for 60 min as previously described for humans. Sperm morphology was again assessed following sperm separation. Using a paired Student’s t test (Microsoft Office Excel 2007, Redmond, WA) the percent of morphologically normal sperm before and after each of the separation methods was compared. Significance was defined as p<0.05.

Results
There was no significant difference in the percent of morphologically normal sperm before separation and following DGCM. However, there was a trend towards a lower percentage of morphologically normal sperm in samples separated using the SUM (see figure below) than in fresh semen (p<0.06) and after DGCM (p<0.09).

Discussion
Equipure™ density gradient centrifugation is easier to perform than other DGCMs reported in dogs because it only requires one overlay layer. In addition, there is no need to perform any additional washing steps after using this method of separation. Ongoing studies are comparing sperm RNA purity and yield between these methods.

References