

Meagan Turner, Mary-Ali Oliver, Dr. Alan Ealy
Virginia Polytechnic Institute and State University, Blacksburg, VA 24061

Abstract

In reproductive research it is important to retrieve the highest number of good quality ovaries to ensure the highest percent of embryos that progress past the blastocyst stage. This experiment was carried out to assess the effectiveness of oocyte retrieval methods relative to ease of use, numbers of each grade recovered, and overall total of COCs from each method. Aspiration and slashing were the two methods of interest. It was hypothesized that slashing would yield the highest amount of COCs overall, but there would not be much difference in grades 1 and 2 between the methods. Trials were carried out four times for each method, with five ovaries per method per trial. The data revealed a significant difference between the totals of COCs retrieved, and the total number of grade 2 COCs. The slashing method had the higher amount in both. This conclusive evidence indicated that the slashing method should be used in more extensive research projects, due to the high yield of good quality COCs.

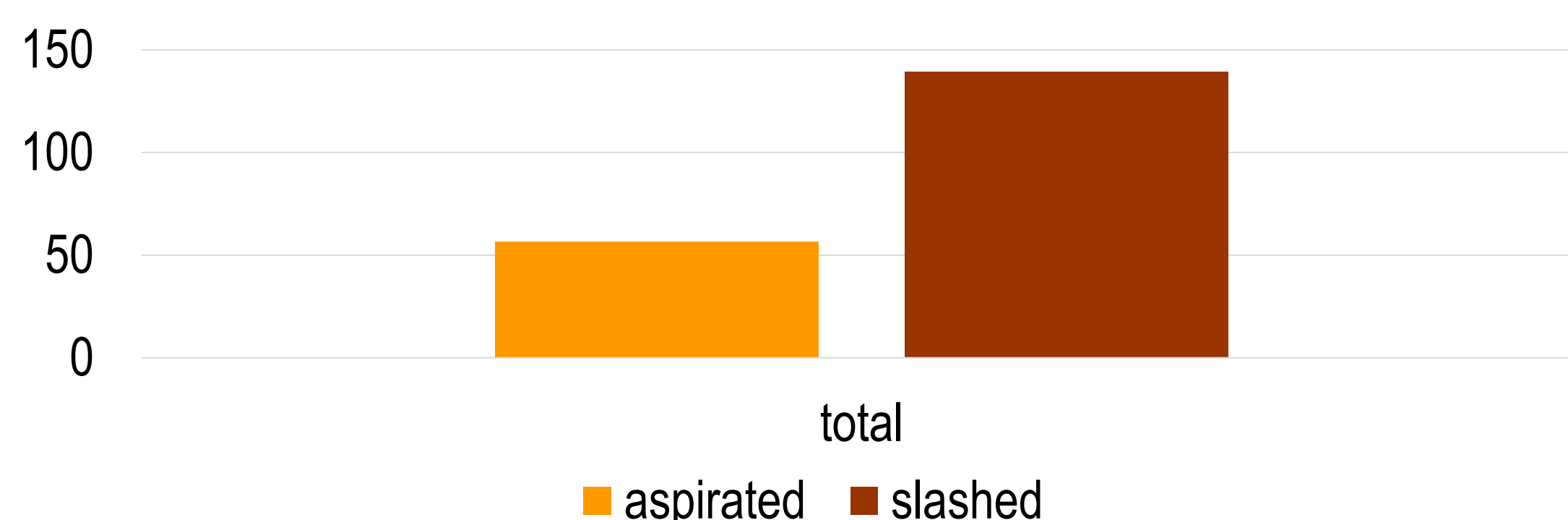
Introduction

Cumulus oocyte complexes (COCs) are used in many areas of reproductive research. Many experimental processes require a high yield of good quality COCs for each round of maturation and fertilization. This ensures a high number of oocytes are fertilized successfully and are viable for further use. To retrieve these COCs, two methods are readily used. These are slashing and aspirating. Typically, the slashing technique is utilized more frequently than aspirating due to the fast turnover rate per slashed ovary. It was hypothesized that slashing would yield the highest amount of COCs overall, but there would not be much difference in grades 1 and 2 between the methods. For this research project the actual yields of COCs for aspirating and slashing were evaluated through four experimental trials. Once COCs were retrieved from each trial, they were separated into their respective grades using a scale of 1-4, 1 being the best and 4 being the worst in quality. The totals of each grade were recorded, and the methods were compared in terms of ease of use, ease of searching, and COC yield.

Materials and Methods

To begin each trial, a syringe with an 18-gauge needle, a scalpel, a beaker with 75mL of media, and a tube with about 40mL of media. Then, ovaries were organized into grades of good, medium, and bad, and 10 ovaries total were taken from either the good category or medium category. The aspiration technique was typically used first; 5 ovaries were gripped at the base in the left hand, and the right hand held the syringe. Each follicle was entered with the needle bevel down, and the follicular fluid was drawn up. Once the syringe was filled, the fluid was expelled into the media filled tube. The process was repeated until all 5 ovaries had been aspirated. The remaining 5 ovaries underwent the slashing process. Holding the ovary with curved hemostats, the scalpel was moved over the entire surface of the ovary making a checkerboard-like pattern and popping the follicles in the process. Once the ovary was completely slashed, it was rinsed in the media filled beaker and “squeegeed” off on the walls of the beaker to remove extra media. This process was repeated until all 5 ovaries were slashed. The tube and the beaker were then moved into the search lab, where the fluids were filtered. The COCs remaining on the filter paper were transferred into a search plate filled with media. The search plates were then searched under a microscope, and COCs were collected using a caprol and transferred to a separate dish. Once both plates had been searched, the COCs were counted and graded in the 2 plates. Totals of each grade were recorded, and notes were made about the search and collection processes.

The Effect of Aspirated vs Slashed Ovaries on Total Number of COCs Retrieved



Results

Trial 1 data	Aspirated	Slashed
Total	35	153
Grade 1	13	19
Grade 2	4	61
Grade 3	5	26
Grade 4	13	47

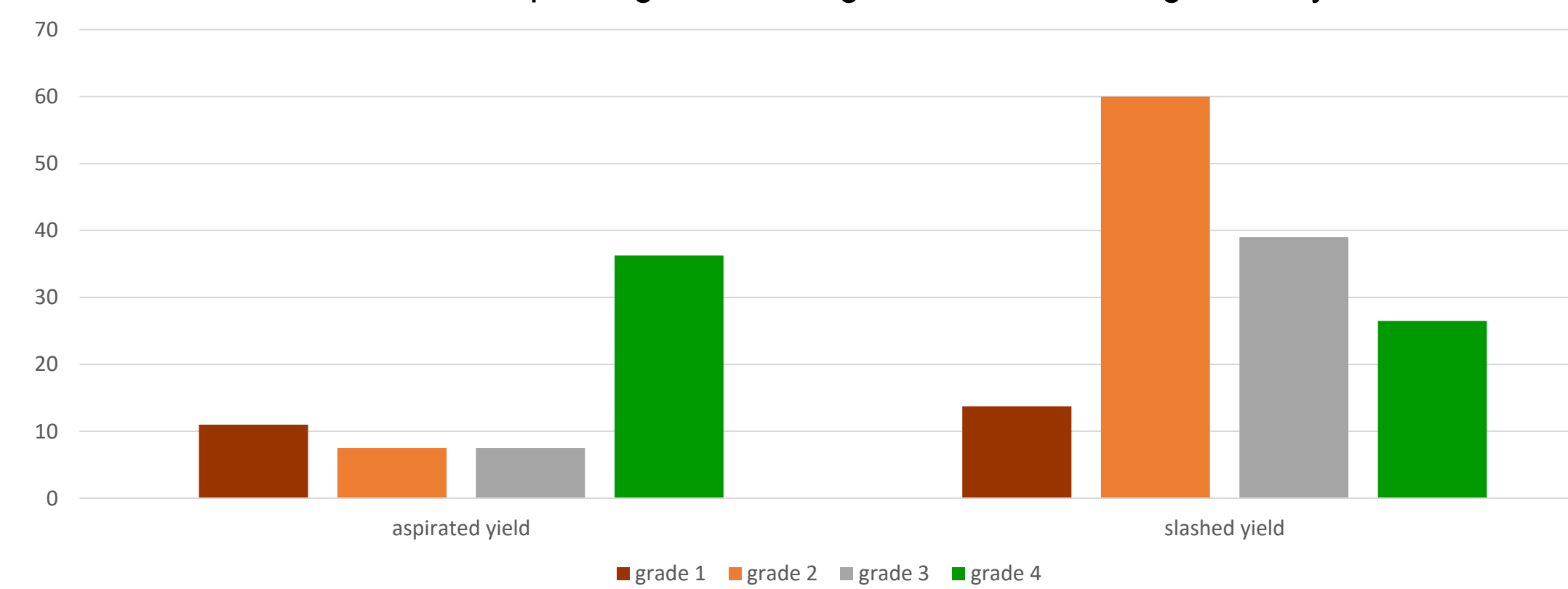
Trial 2 data	Aspirated	Slashed
Total	66	115
Grade 1	5	16
Grade 2	11	52
Grade 3	13	31
Grade 4	37	16

Trial 3 data	Aspirated	Slashed
Total	76	208
Grade 1	2	11
Grade 2	9	94
Grade 3	7	68
Grade 4	58	35

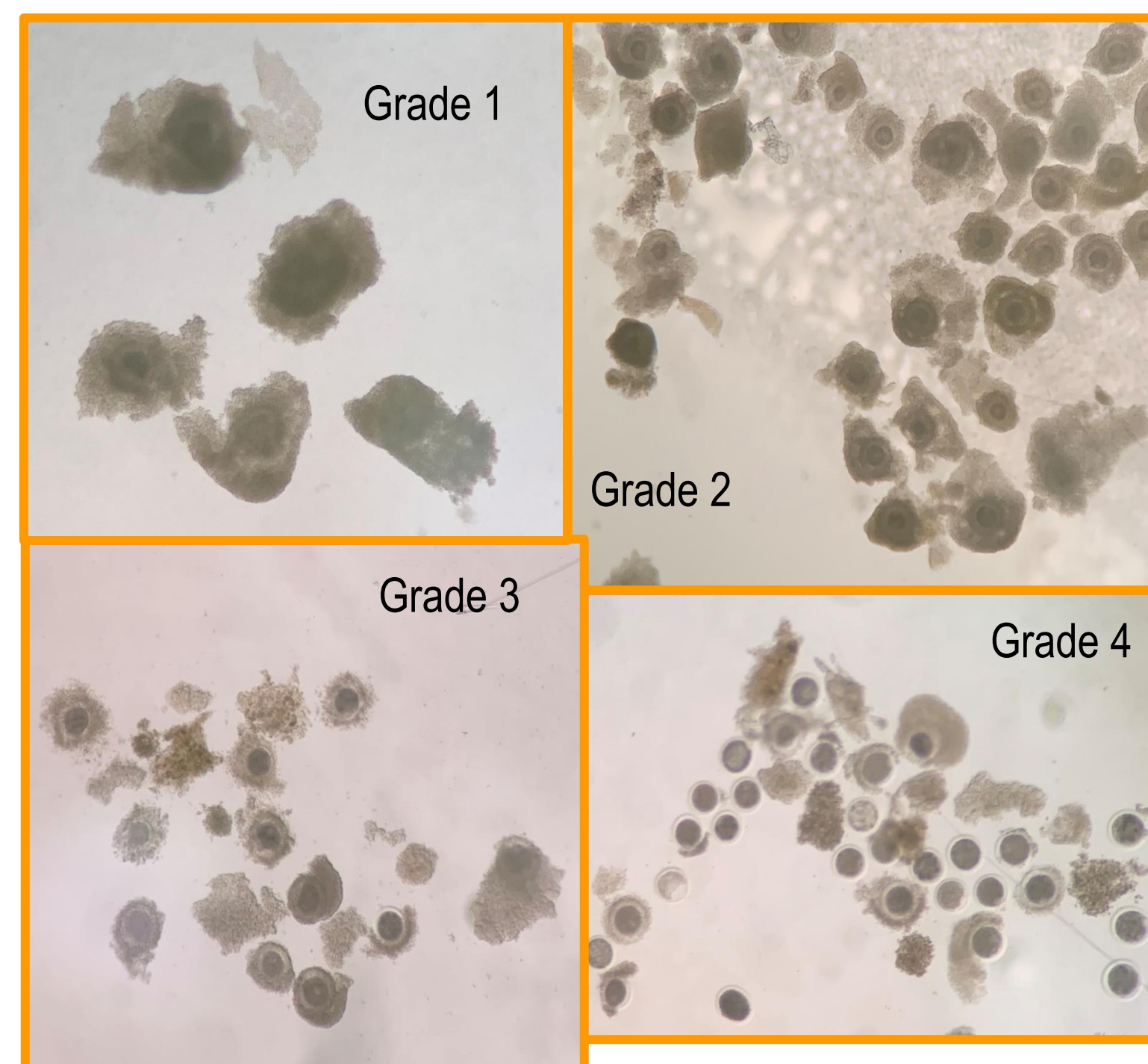
Trial 4 data	Aspirated	Slashed
Total	50	81
Grade 1	2	9
Grade 2	6	33
Grade 3	5	31
Grade 4	37	8

Description: tables above represent the data of each grade of COC from each method taken over the 4 days of trials

The Effect of Aspirating vs Slashing Ovaries on average COC yield



Description: graph compares the effect of retrieval method on average COC yield in each grade. Note the higher amounts of grade 1 and 2 in slashed ovaries, and higher grade 4 in aspirated ovaries



Description: notice the difference in cumulus cell accumulation and nuclear degeneration as COC grade worsens, specifically in grades 3 and 4



Top left and right: picture of the aspiration process, showing the entry point of the needle into the ovary to extract follicular fluid. Hemostats or hands may be used to grasp the ovary; shown here are hemostats to allow for better viewing



Bottom left and right: pictures of the slashing process, showing positioning of scalpel to extract follicular fluid into beaker below. Hemostats grasp onto the ovary to allow for freedom of movement and decreased incidence of injury



Discussion

The yield of COCs from aspirated ovaries was consistently lower overall, along with a higher average of grade 4 in the totals. The yield of slashed ovaries displayed not only a higher overall number, but a higher number of grade 1 and 2 COCs, which are typically used in further research according to T.C. Wood, “Nearly twice as many grade 1 oocytes matured and more than twice of these were fertilized compared with grade 2 oocytes...Oocytes from grade IV COCs were poor in terms of maturation (compared with grades I to III) and fertilization (compared with grades I and II).” In their research, it was found that although a higher number of grade 3 and 4 COCs were used in the experimental group, none of them survived to the blastocyst stage, whereas 8/123 grade 1 and 5/226 grade 2 COCs hatched from the blastocyst stage. Grade 3 and 4 COCs do not typically move on to be matured and fertilized due to their high rate of cellular death in vitro; they are often already terminated as seen by their denuded centers. Some drawbacks to each method were noted, although those of aspiration outweighed those of slashing. In performing aspiration of ovaries, it was noticed that almost double the time per ovary was necessary to thoroughly extract all follicular fluid. Working the syringe was also slightly difficult with one hand. Slashing is a much quicker process, and as summarized in the data, results in a higher yield. The quicker retrieval time does not make for a quick search time. When looking under the microscope, more ovarian material shields from a good view of all available COCs and could impact the total realized number. However, this issue did not carry over to the data as a higher number of COCs was realized from slashing. It was concluded that the slashing technique is superior to aspiration for the overall higher yield of good quality oocytes, ease of use, and quicker retrieval time. Though all these reasons contribute to the superiority of the slashing method, in practice a higher number of good quality oocytes is of highest priority. This is because research performed must have many trials with large experimental groups to account for possible cell death that may occur. According to Luis Aguila, The efficiency of producing embryos using in vitro technologies in livestock species rarely exceeds the 30–40% threshold, which indicates a majority of in-vitro fertilizations are not carried to completion. Therefore, measures must be taken to account for the undoubtable cell death and increase experimental group number.

References

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- Luis Aguila, F. T., Jacinthe Therrien, Ricardo Felmer, Martha Valdivia and Lawrence C Smith. (2020). Oocyte Selection for In Vitro Embryo Production in Bovine Species: Noninvasive Approaches for New Challenges of Oocyte Competence. MDPI, 10(2196). doi:10.3390/ani10122196