

Sexing Through Spinning

What are your chances of getting a filly? What are my chances of getting a colt?

1. All females carry solely the “X” Chromosome in their DNA.
 - a. The “X” Chromosome is $2/3^{\text{rds}}$ the size and weight of the “Y” Chromosome.
2. All males carry both the “X and the Y” Chromosome in their DNA.
 - a. All sperm being of single DNA are either “X or Y” dominant.
 - i. The “X” Chromosome is $2/3^{\text{rds}}$ the size and weight of the “Y” Chromosome.
 - ii. The “Y” Chromosome is $1/3^{\text{rd}}$ the size and weight of the “X” Chromosome.
 - b. When fertilization takes place, either the “X” sperm cell or “Y” sperm cell, dictates whether or not the resulting conceptus will be a male ♂ i.e.: “XY” or a female ♀ i.e.: “XX”.
3. For best results, use a 15 ml graduated polypropylene centrifuge tube as it has a relatively steep taper; a centrifugation cushion such as Opti-Prep helps to protect the sperm cell from damage.
 - a. Using this type of tube allows the sperm pellet to be spun down into a longer column rather than a flat coin shape found when using a 50ml or large diameter tube.
 - b. If the pellet is, too flat it becomes extremely difficult to obtain the bottom portion where the “female X” sperm will ultimately settle.
4. When spinning the collection down, it is best to centrifuge at 500 g for 12 to 14 minutes.
 - a. always be sure to filter the semen in a Next Generation Semen Gel Filter
5. The spinning rate should remain at 500 g for 12 to 14 minutes
 - a. The Next Generation 15 mL centrifuge calibrates 487 g @ 2000 RPM
 - i. If you spin the dose faster, you are very likely going to crush or damage a high percentage of the “female X” sperm.
 - ii. Any slower and you will not get a good sperm pellet. The centrifugal force will position the sperm cells accordingly:
 - b. The “X” sperm go to the bottom of the test tube because of its weight i.e. $2/3$ heavier than the “Y”.
 - c. The “Y” sperm cell will sit a top the “X” sperm cells because of its lighter mass i.e. $1/3$ lighter than the “X”.
6. If you want to test to see if you have spun it long enough or too long, make a slide of the seminal fluid after your initial spin down.
 - a. Technically, you should have half of the sperm left in the seminal fluid.
7. In order for a nice pellet to form, you can then ¹extract the pellet according to your specific sexing requirements using a pipette or by carefully pouring off the top seminal fluid and then extend the remains.

¹ Be careful when penetrating the pellet because obviously you are going down into the pellet and can trap material from the top in the pipette if your technique is not careful which would defeat the propose of separating the “X” form the “Y” marked sperm.

The Best Opportunity For A Filly:

Draw off the seminal plasma with a pipetter and then carefully draw off approximately the top 1/3 of the sperm pellet left in the test tube and discard. The pellet is not hard, it is more a sand like object. You will stir up the pellet, once you penetrate it. I typically obtain ¾ to 1 ml of raw sperm to extend which is ~500 million. Reconstitute the remaining pellet in the bottom of the test tube with fresh semen extender. Your insemination dose should remain the same i.e.: 750 million progressively motile sperm extended 5:1. It is then suggested, that a horn style insemination occur so that you place the physical insemination dose at the tip of the uterine horn, your opportunity to achieve a female has now increased to a 4:1 ratio with given tolerances included.

The Best Opportunity For A Colt:

Draw off the seminal plasma with a pipetter and then carefully draw off approximately the top 1/3 of the sperm pellet left in the test tube. Reconstitute that 1/3rd pellet with fresh semen extender in a separate centrifuge tube. Remember, the pellet is not hard it is sand like and you will stir it up once you penetrate it. Typically, ¾ to 1 ml of raw sperm is ~500 million sperm cells. Your insemination dose should remain the same i.e.: 750 million progressively motile sperm extended 5:1. It is then suggested, that a horn style insemination occur so that you place the physical insemination dose at the tip of the uterine horn, your opportunity to achieve a male has now increased to a 4:1 ratio with given tolerances included.

8. Once you have the sperm you wish to extend for your specific purposes, you can then begin to process it like any other sample, but keep in mind that it is raw concentrated sperm.
9. Use the Next Generation Quick Check to analyze and procure your insemination dose
10. 15 ml of raw sperm will yield about a 1.5ml sperm pellet with a usual count of 750 million progressively motile sperm, although, when shipping a physical sperm count should be allotted for every mare.

A couple of footnotes:

- 1) If this dose is to be inseminated by means of a deep horn procedure, do not extend.
- 2) If you are inseminating conventionally, extend at 25 million/mL o 25ml.