Chapter 18

Laboratory Methods of Achieving Human Sex Preselection

J. H. Check, K. Nowroozi, S. O. Cooper, B. S. Shanis, and A. Bollendorf

Two primary laboratory methods have been designed to achieve human preconception sex selection. They are (1) enriching for an increased Y-bearing sperm percentage and (2) enriching for increased X-bearing sperm percentage. Over a period of years, several techniques have been developed for use with each of these methods (Table 18-1).

ENRICHING FOR INCREASED Y-BEARING SPERM PERCENTAGE

Albumin Separation Technique

The majority of techniques employed with this method involve trying to separate X-bearing from Y-bearing spermatozoa based on some distinguishing physical property. The Albumin Separation Technique was one of the first methods that attempted to increase the percentage of Y-bearing sperm. As reported and described by Ericsson, this technique is based on the assumption that Y-bearing sperm swim faster than X-bearing sperm.[8] The procedure involves migration of the sperm through progressively denser solutions

Table 18-1 Clinically Tested Separation Methods and Pregnancy Rates

Technique	Pregnancy rate
Y-bearing sperm percentage	
Albumin separation	80%
	82.6% (three layer)
	73%
Swim-up (in vitro)	90.9%
Swim-up (in vivo)	86%
X-bearing sperm percentage	
Albumin separation	74%
Sephadex gel-filtration	72.7%
Discontinuous Percoll density gradient	68.9%

of bovine serum albumin (BSA). The BSA is subsequently changed to human serum albumin (poor salt) in concentrations of 7.5%, and 12%, and 20%. It has been claimed that a concentration of Y sperm of as high as 85% (as determined by quinacrine staining of the long axis of the Y chromosome) in the 20% fraction have been made.[17] The hypothesis is that the Y-bearing sperm has a greater ability to penetrate an interface between liquids and between continuous gradients of an isolation medium. This procedure is patented by Gametrics, Ltd.

Corson et al., using the albumin separation technique, reported that 28 males were born out of 35 conceptions (80%).[6] A male-birth ratio of 73.5% (39 males and 16 females) using a three-layer separation was reported by Beernik and Ericsson.[1] However, when the females who had taken clomiphene citrate were excluded, there was a male-birth ratio of 82.6% (38 males and 9 females). The two-step, two-layer separation technique was reported to produce a male birth ratio of 73% (19 males and 7 females).

Swim-Up Technique

Employing the swim-up technique, Rawlins et al. presented data suggesting an increased rate of male offspring in patients who conceived by in vitro fertilization (IVF).[15] Following fluorochrome quinacrine mustard staining, the initial specimens averaged 47% Y body, but following treatment they averaged 60% Y-bearing sperm in the lower portion and 36% in the upper fraction. Check et al.[5] also claimed this technique was effective for increasing male offspring in women taking ovulation inducing drugs following both intracervical and intrauterine insemination.

In Vitro Method

Rawlins' method involves placing semen into 15 mL centrifuge tubes in 0.25 mL aliquots.[15] The aliquots are washed 2 times using 0.5 mL Ham's F-10 medium and 10% maternal serum and then centrifuged at 200 × g for 10 minutes. The final pellets are overlain with 0.5 mL medium and incubated at 37°C in 5% CO_2 for 1 hour. Following incubation, the first 0.25 mL are discarded and the final 0.25 mL is available for insemination of the oocytes (Figure 18-1).

In the method described by Check et al.[5] the semen was diluted in equal parts with Ham's F-10 medium and 0.5 mL aliquots are placed into 1.5 mL centrifuge tubes. The tubes are then layered with 0.5 mL Ham's F-10 medium and centrifuged for 5 minutes 500 × g. This is followed by a 1 hour incubation at 37°C in 5% CO_2. The supernatant was then diluted in equal parts with medium and centrifuged for 5 minutes. The supernatant is discarded, and the pellet was resuspended in 1 mL media and recentrifuged. The final pellet was resuspended for insemination (Figure 18-2).

Rawlins et al. reported a male-sex ratio of 84.6% (11 males and 2 females) and a live-birth ratio of 90.9% following swim-up in IVF pregnancies.[15] Check et al. report data on the sex ratios following swim-up in 10 women treated with human menopausal gonadotropins (hMG) and 9 mothers with clomiphene citrate.[5] Nineteen of 22 births (86%) were males.

Convection Counter Streaming Galvanization Technique

The convection-counter streaming galvanization technique was described by Daniell et al.[7] An electrical current is passed through the sperm. This resulted in 77% Y-bearing spermatozoa at the anode end. This technique, however, has not yet been clinically evaluated.

ENRICHING FOR INCREASED X PERCENTAGE

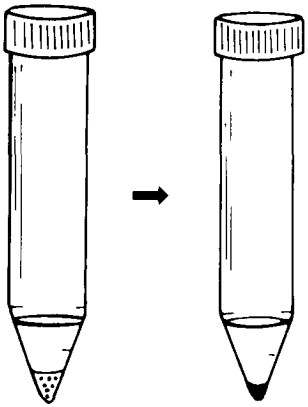
Albumin Separation Technique

Albumin separation technique, method of sex preselection, is basically the same as that franchised by Gametrics, Ltd., for increasing the percentage of Y offspring and is also patented for increasing female births. The only real difference between the techniques used for male and female patients is that the female is treated with clomiphene citrate. Presently, there is no known explanation for the skewing of the sex ratios. The discovery was apparently made inadvertently by noting a lower percentage of male neonates in some series in which a large proportion of the females had been receiving ovulation-inducing drugs when the albumin separation technique was used for Y-bearing sperm enrichment.

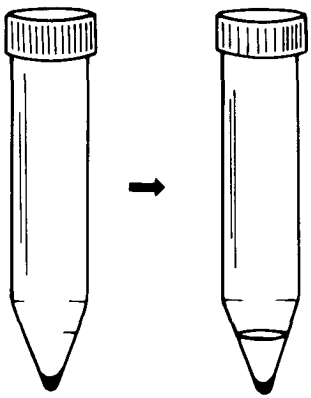
The separation technique for the female is very similar to Ericsson's male method. This procedure is patented by Gametrics, Ltd.

Male Sex Selection
Swim-Up Technique

In-Vitro Method



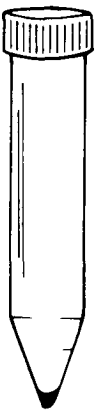
1) Add 0.5 ml Ham's F-10 to 0.25 ml aliquots of semen and wash 2 times by centrifuging at 200 x g for 10 minutes.



2) Remove supernatant and overlay sperm pellets with 0.5 ml medium.

3) Incubate at 37°C in 5% CO_2 for 1 hour.

4) Discard top of 0.25 ml of the overlay.

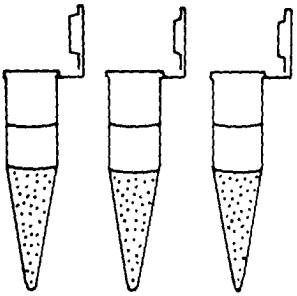


5) Remove bottom 0.25 ml of the overlay and use for insemination.

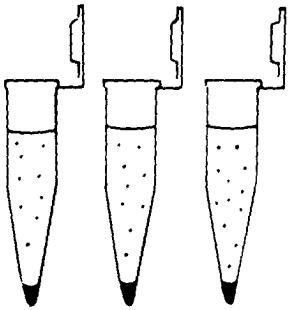
Figure 18-1

Male Sex Selection
Swim-Up Technique

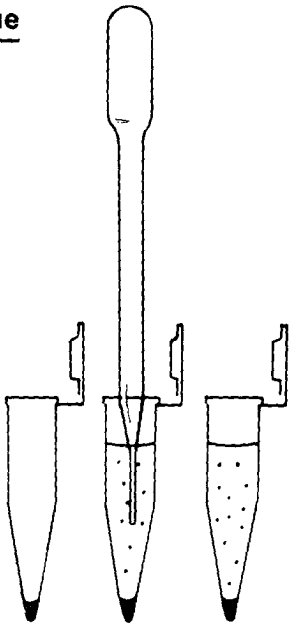
In-Vivo Method



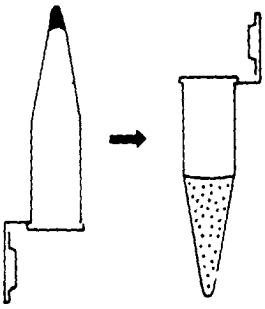
1) Dilute the liquefied semen 1:1 with Ham's F-10 media. Divide into 1.5 ml centrifuge tubes containing 0.5 ml of the semen mixture. Then layer with 0.5 ml media.



2) Centrifuge for 5 minutes at 500 x g. Then incubate at 37°C in 5% CO_2 for 1 hour to allow the motile sperm to swim up.



3) Remove all supernatants and dilute 1:1 with Ham's F-10 media. Centrifuge for 5 minutes at 500 x g.



4) Decant supernatants; resuspend with 1 ml media and centrifuge for 5 minutes at 500 x g. Decant supernatants and resuspend pellets for insemination.

Figure 18-2

Female-Preselection Technique

There are no published data controlled or uncontrolled on the efficacy of the female-preselection technique. A 74% female rate in patients treated with the "modified" technique for female selection was reported by Gametrics, Ltd., in October, 1987.

Sephadex Gel-Filtration Method

Steeno et al. reported that when they passed sperm through a gel bed of Sephadex in an attempt to improve motility, they found by Quinacrine staining that the filtrate contained predominately X-bearing sperm while the Y-bearing sperm were retained on the gel column.[16] The gel filtration with Sephadex is based on the separation of large molecules from smaller ones. Smaller molecules are easily trapped in a network of dextran cross-links, while larger ones can pass through this mechanism. The authors were unable to hypothesize the mechanism responsible for preferential absorption of the Y-bearing sperm to the column.

The technical details involve diluting the semen in equal parts with Hepes' buffered Hank's solution and centrifuging at 500 × g for 10 minutes. The supernatant is discarded and the pellet is resuspended in 1 mL Hank's solution. Sephadex G-50 fine, swollen overnight in Hank's solution, is used. A 0.9 × 23 cm glass column is half filled with the Sephadex and 20 mL of Tyrodes solution is passed through the gel column. Next, the sperm suspension is added on top of the gel beads and followed by 9 mL of Hank's solution. The voided specimen is then centrifuged and the pellet resuspended for insemination (Figure 18-3).

Corson et al.[6] reported 8 female births of 11 total births (72.7%), despite the fact that 9 of the women had previously delivered 15 children all of whom were males.

Discontinuous Percoll Density Gradient

The discontinuous Percoll density gradient method described by Kaneko et al.[14] separates X-bearing and Y-bearing sperm by means of discontinuous Percoll density gradient. (An 82% X-bearing sperm population in the bottom fraction of an eight-layer discontinuous gradient was reported.)[14] Increasing the number of steps of the gradient to 12 improved the purity of X-bearing sperm to 94%. The sedimentation velocity of human X-bearing sperm was revealed as being faster than that of Y-bearing sperm, despite the fact that the apparent densities of the two types of cells obtained by equilibrium sedimentation were similar.

With this technique the semen specimen is diluted 1 : 1 with Hepes' buffered Hank's solution and layered onto a 15 mL centrifuge tube, which is filled with increasingly dense layers of isotonic Percoll. The tube is centrifuged at 600 × g for 30 minutes. The top layers of Percoll are discarded and the bottom layer is washed with an equal volume of Hank's solution and centrifuged at

Female Sex Selection

Sephadex Gel Filtration Method

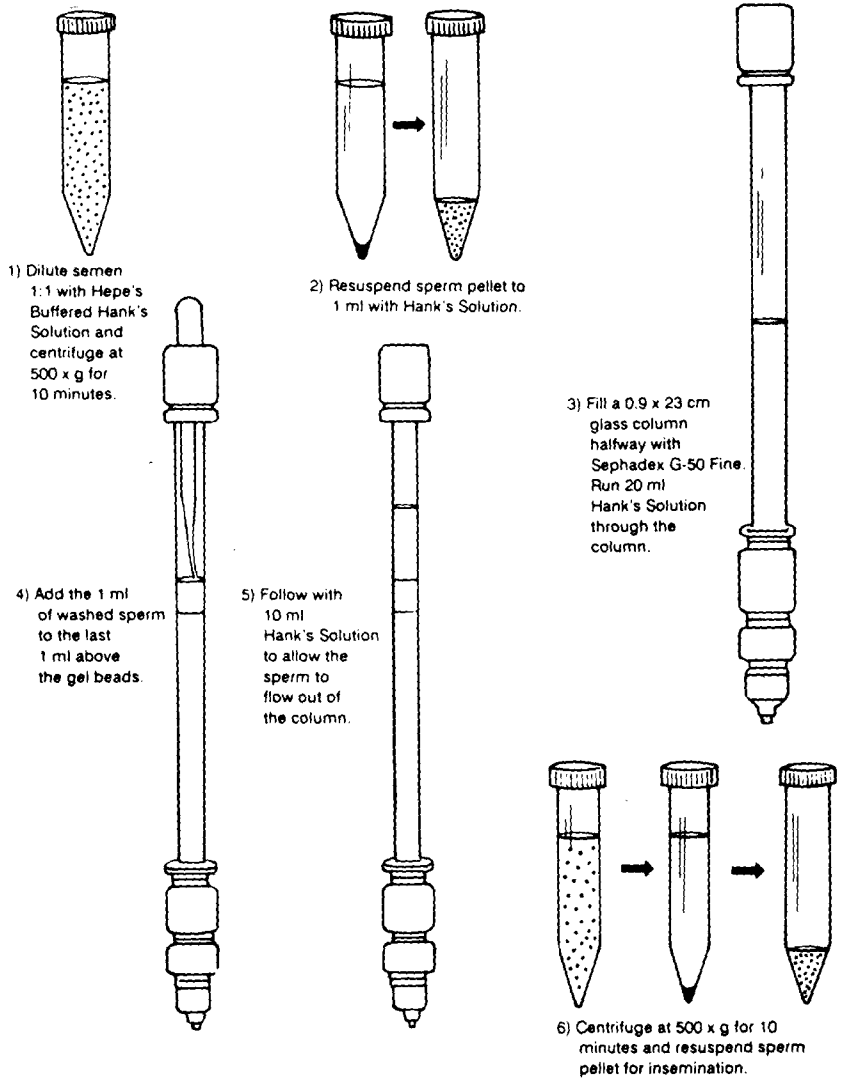


Figure 18-3

500 × g for 30 minutes. The supernatant is discarded, and the final pellet is resuspended for insemination (Figure 18-4).

Iizuka et al. reported 31 females out of 45 births (68.9%) following insemination after separation using discontinuous Percoll gradient separation.[9,10]

Other Techniques

Many other techniques have not yet been clinically tested. They include the convection counter streaming galvanization technique, in which Daniell et al.[7] reported an average of 76% X-bearing spermatozoa, and the free-flow electrophoresis method, in which Kaneko et al. reported separation of human X-bearing and Y-bearing spermatozoa using free-flow electrophoresis.[14]

This basic method has three different procedures. The more layers that are used, the higher the amount of X-bearing sperm that can be recovered.

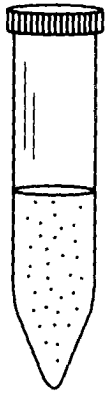
1 One layer: 20% Percoll layer at 300 × g for 15 minutes
2 Four layer: 80%, 72%, 64%, and 50% at 600 × g for 30 minutes
3 Twelve layer: 30% to 80% Percoll in 5% steps at 250 × g for 30 minutes

First the whole specimen is washed and concentrated by layering 2 mL semen onto 4 mL of 25% Percoll and centrifuged at 250 × g for 20 minutes. The sperm pellet is resuspended in 25% Percoll and layered on top of the 12 layers.

VALIDITY OF Y-BEARING TECHNIQUES

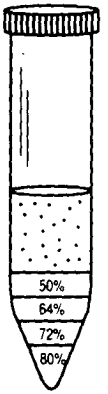
There have been several criticisms of the albumin-separation technique. The main criticism was that the procedure was not controlled. The fluorescent "Y body" acrosin technique may falsely increase the Y-bearing-sperm count because of nonspecific fluorescence of autosomal chromosomes.[10] The current test of preference is the use of DNA probes to determine Y chromosomal DNA. Preliminary data with DNA probes has failed to confirm separation of X-bearing from Y-bearing sperm by albumin density gradients.[3] Brandriff et al.,[2] using a modification of the zona-free hamster-egg-penetration test designed to obtain sperm karotypes, failed to show Y-bearing sperm enrichment and, in fact, showed a slight predilection for X-bearing sperm enrichment following separation with albumin gradients. Nevertheless, even if the initial claims of Y-bearing sperm enrichment by the Ericsson method are found to be in error, the possibility still remains that an increase in male progeny may exist by somehow damaging or impairing fertilization of the X-bearing sperm.

Female Sex Selection
Percoll Method

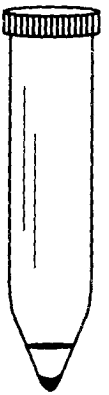


1) Dilute semen 1:2 with Hepe's Buffered Hank's Solution.

2) Layer 1ml of each Percoll Density Layer- starting with the highest density.

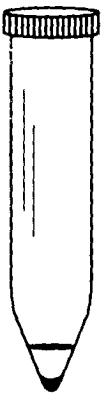


3) Layer diluted semen on top of Percoll layers and centrifuge at 600 x g for 30 minutes.



4) Remove all layers except the bottom 1ml 80% layer.

5) Dilute the 80% layer 1:2 with Hepe's Buffered Hank's Solution and centrifuge at 600 x g for 10 minutes.



6) Remove supernatant and wash again to remove all traces of Percoll. Then resuspend sperm pellet for insemination.

Figure 18-4

One of the main objectives to the Ericsson method is that it is franchised by Gametrics, Ltd., and is therefore expensive. Thus, if the swim-up techniques prove effective in controlled studies for increasing male births, patients will be able to choose a nonpatented, less expensive technique. One can similarly criticize Rawlins' conclusions about separating an upper fraction of 36.1% Y-bearing sperm versus 60.4% Y-bearing sperm in the lower fraction compared to 47.1% Y-bearing sperm in the initial specimen. Rawlins also used the fluorochrome quinacrine mustard stain to stain the sperm.[1] In addition, Rawlins' studies involved patients undergoing IVF.

The albumin-separation technique looses its effectiveness when the females are treated with ovulation-inducing drugs. Thus, one factor indicating the possible effectiveness of the swim-up technique is that the studies by Rawlins et al.[15] and Check et al.[5] (in press) both employed women taking ovulation-inducing drugs, the former using hMG exclusively and the latter employing either clomiphene citrate or hMG. Despite this, both groups showed a comparably high percentage of male offspring. Actually, there are no reports of the efficacy of male gender preselection following swim-up techniques in patients not taking fertility drugs.

With the exception of minor differences, the basic swim-up techniques employed by Rawlins et al. and Check et al. were similar. Despite using a swim-up technique for our IVF cases, the first 10 deliveries to date were split equally between males and females (J.C., unpublished data). However, the swim-up technique used in IVF differs considerably from the technique used in vivo both for intracervical and intrauterine insemination. The IVF swim-up technique yields lower-motile sperm recovery rate than in vivo techniques.

We conducted a simple experiment to try to determine why different swim-up methods may give discordant results. Following washing of the sperm and gentle centrifugation at 250 × g, we found an average of 60% Y-bearing sperm (using quinacrine fluorescent staining) in the supernatant and 40% in the pellet. Following the swim-up technique, the ratios of Y-bearing to X-bearing sperm became almost equal (51% to 49%). For IVF, the supernatant is discarded, thus losing a high concentration of Y-bearing sperm, although apparently more Y than X will swim-up because the pellet starts out with a higher percentage of X-bearing sperm. The supernatant is not discarded in our non-IVF-washing techniques, and this, with a high Y-bearing sperm concentration, is added to the swim-up collection with its equal X-bearing and Y-bearing sperm, thus shifting the balance to a Y-bearing sperm predominance.

The higher the centrifugation speed and the smaller the volume, the greater is the number of sperm that will enter the pellet and the less that will go into the supernatant. Thus variations in these parameters may affect the results of sex selection. Perhaps an even higher percentage of male births can be achieved by performing intracervical inseminations with only the initial supernatant.

VALIDITY OF X-BEARING TECHNIQUES

Until more clinical data are available, it is difficult to decide whether Sephadex chromatography or Percoll density gradient (either the monolayer or discontinuous four-step method) will prove more effective for X-bearing gender preselection. However, because the specimen used for insemination is freer of debris and bacteria with the Percoll technique, this is the more preferred procedure if intrauterine insemination is contemplated. Because the pellet following simple centrifugation during the washing procedure seems to have a higher percentage of X-bearing sperm in our preliminary experiments, it may be interesting to see if a higher female-birth rate may be achieved by merely resuspending the pellet without swim-up.

It should be emphasized that our experiments on swim-up techniques also involve the quinacrine fluorescence technique, which is being challenged for its accuracy. Our only question of the accuracy of this technique is: Why does our female-sex selection yield a high percentage of X-bearing sperm when nonspecific fluorescence of autosomal chromosomes should yield a falsely higher Y-bearing sperm percentage? Perhaps this technique is even better for X-bearing sperm enrichment than it appears because the Y-bearing sperm percentage may be falsely increased. Or perhaps some artifact in the "more accurate" DNA probes leads to falsely lowered Y percentage.

ACKNOWLEDGMENT

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