

## MALE:FEMALE SEX RATIO IN BIRTHS RESULTING FROM IVF ACCORDING TO SWIM-UP VERSUS PERCOLL PREPARATION OF INSEMINATED SPERM

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Two centers have independently reported a higher rate of male to female births following insemination of sperm prepared by a modified swim-up technique. The principle of the modified swim-up is that a small percentage of the x-bearing sperm are the fastest and travel to the top of the supernatant, followed by the y-bearing sperm; the bulk of the x-bearing sperm remain in the pellet. In this technique, the very top layer is discarded and the resulting supernatant is collected, leaving only the pellet. In contrast, with the conventional swim-up technique, the entire supernatant is collected. The study presented herein retrospectively evaluated the male to female sex ratio of births from in vitro fertilization using standard swim-up technique and compared these results to the ratio obtained from separating with Percoll. There were 53% male births with swim-up vs. 54% with Percoll in singleton pregnancies and 51% males with swim-up vs. 40% with Percoll with multiple births. Thus, conventional swim-up alone does not increase percentage of male births.

**Keywords** male, female, IVF, sperm, insemination

Several methods of separating sperm from seminal plasma have claimed an increased percentage of y-bearing sperm. One such method is the albumin separation technique, reported by Ericsson et al. [4]. This method uses columns with various concentrations of human serum albumin. Using a three-layer technique a 73.5% male birth rate has been reported [1]. Certain types of swim-up procedures have also claimed to increase the percentage of male offspring. Rawlins et al. found that pregnancies achieved by in vitro fertilization (IVF) with spermatozoa

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prepared by the swim-up method resulted in 10 of 11 male births (90.9%) [6]. Similarly, Check et al. reported 86% male births following swim-up separation in in vitro pregnancies [2]. In a prospective study employing a modified swim-up technique, 88.5% male offspring were reported; this is compared to 50% pregnancies following intrauterine inseminations (IUI) with Percoll prepared sperm and 52% in the untreated control group [3]. The retrospective study presented herein evaluated the male birth ratio achieved by IVF in sperm separation by standard swim-up method versus Percoll preparation. The swim-up method is different than the one standardly used to prepare sperm for IVF. The hypothesis was that the conventional method should not increase the percentage of male births.

## MATERIALS AND METHODS

In this study a standard swim-up method and a Percoll discontinuous gradient were used. Regardless of the type of separation technique used, the semen was collected and allowed to liquefy for 20 min. The counts, motilities, and grades of motilities were subsequently performed.

*Standard Swim-up.* The semen was diluted 1:3 with Hams F-10 bicarbonate in the standard swim-up procedure and washed 2× by centrifuging at 300g for 5 min. After the second wash, the supernatant was removed and the pellet was carefully overlaid with 0.5 mL of media, placed in a slant rack at a 20° angle, and allowed to incubate for 1 h at 37°C with 5% CO<sub>2</sub> and 95% air. After 1 h approximately 300–500 µL was removed and used for insemination of the eggs.

*Percoll Discontinuous Gradient.* The semen was diluted 1:2 with modified human tubal fluid (HTF). Up to 2 mL was layered into a column containing 1 mL each of 90, 60, and 45% Percoll, respectively. The columns were centrifuged at 250g for 20 min. The semen layer was removed and discarded. The layers were penetrated with a pipet and the bottom pellet (up to 1 mL) was withdrawn from the sample. The pellet was washed twice with 1 mL of media at 250g for 5 min and resuspended in 0.2 mL of media.

## RESULTS

One hundred and three IVF births resulted from swim-up sperm preparations between 19 March 1988 and 21 June 1991; there were 82 singleton pregnancies, and 43 of 82 (52.4%) were male. There were 43 babies resulting from multiple births, of which 51.2% were male (Table 1). Percoll preparations were used less frequently. There were 34 births between 1

TABLE 1 Pregnancy Outcome by Sperm Preparation Used

Outcome	Swim-up	Percoll
Singleton, male	43	14
Singleton, female	39	12
% Male (singleton)	52.4	53.8
Twins, 2 males	6	2
Twins, 2 females	5	3
Twins, male and female	9	1
Triplets, 2 male, 1 female	0	1
Triplets, 1 male, 2 female	1	1
% Male (multiple births)	51.2	40.0

October 1990 and 27 January 1992 using the Percoll procedure. Of the 26 singleton pregnancies in this group, 53.8% were male. There were 18 births resulting from multiple gestations, and 40% of these were male (Table 1).

## DISCUSSION

The differences between the standard swim-up technique used to prepare sperm for IVF versus the modified swim-up technique for male sex preselection are as follows: (1) The sperm was first separated from the seminal plasma in the standard technique; however, the sperm remained in the seminal plasma in the modified procedure. (2) The standard used 1–2 15-mL centrifuge tubes, the modified used several microcentrifuge tubes. (3) In the standard protocol the tubes were placed in a 20° angled slant rack, whereas in the modified technique the tubes were not slanted. Most importantly, when the modified swim-up for sex selection is used, the very top layer of the supernatant is discarded because a small percentage of the x-bearing group are actually the fastest and thus reach the top first. However, the majority of the x-bearing sperm remain in the pellet, so the aliquot that is used avoids not only the top portion but does not take the sample too close to the pellet.

There are great similarities between the swim-up of Rawlins et al. and the independently designed modified swim-up technique of Check et al. There may well be advantages for preparing a better sperm specimen for either IUI or IVF using similar techniques to the modified procedure versus the standard procedure, in that the centrifugation of the specimen in the standard swim-up may release harmful oxidants from the leukocyte or damage sperm cells [5]. However, the patients should be cautioned about a possible skewing toward male births.

The IVF center is presently using the Percoll separating method for IVF. Plans are being made to ask patients if they have a preference for sex of offspring and those preferring males will have the sperm prepared by the modified swim-up technique and a comparison will be made to prospectively match IVF patients whose sperm were prepared by Percoll discontinuous gradients.

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