SPERM SEPARATION PROCEDURES IN HUMAN ART

Safety and efficiency

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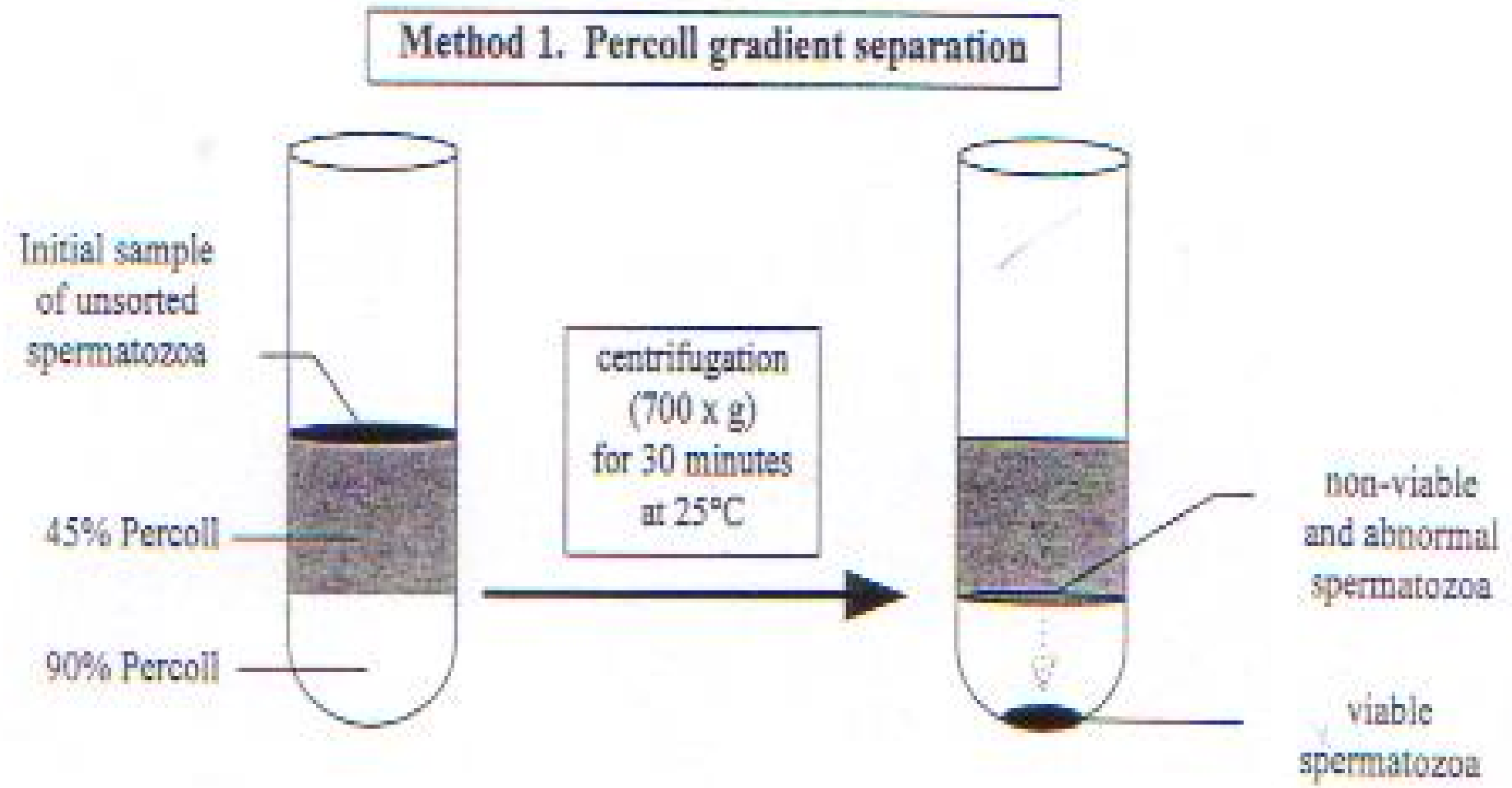
Percoll & Swim Up – Basic procedure

Mc Evoy T. et als – MRT I Class Handout



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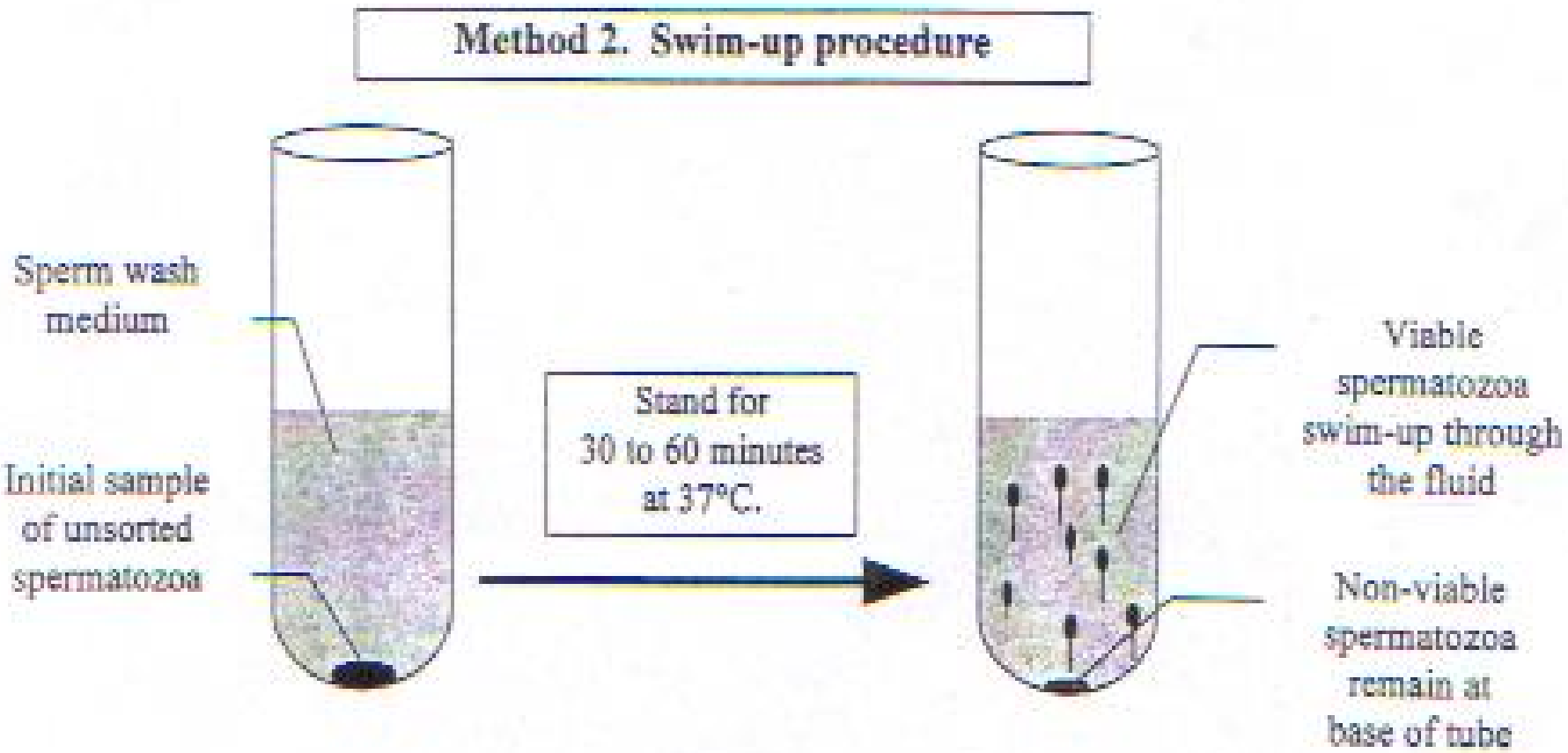

Percoll - Basis

- Percoll and its contemporaries do more than just separate motile from non-motile sperm: they impact on capacitation.

- Biological basis for using the gradient preps: reduces the generation of ROS oxygen radicals, selects for the best fraction of motile and normal sperm.

Swim-up - Basis

Tip

- **Harvest carefully the middle layer** in the post swim up sample as the sperms just above the pellet might have abnormalities (not able swim up better)

- Also: Sometimes top layer usually contains dead sperms due to air media interface.

Percoll - The controversy

- **Many variations of Percoll gradients**, not all will produce similar sperm populations.

- Some older variants did seem to induce detrimental effects on the prepared sperm (due to other, extraneous factors, such as osmotic stress, lack of protein, etc)

Percoll osmolarity

- **Isotonic for seminal sperm is NOT 285 mOsm/kg**, (what all typical culture media are designed to be).
- **Osmolarity of liquefied semen is well over 300**, typically about 340 mOsm upon completion of liquefaction
- Consequently, sperm experience an osmotic shock in passing from semen layer into Percoll solutions, and when resuspended from pellet at bottom of gradient.
- **Osmotic shock signs**: substantial changes in their movement from rather "shaky" pattern to more highly progressive pattern (with shift into hyper activated patterns occurring as well).
- **Recommendation (1997):** use of hypertonic Percoll gradients, with gradient layers prepared using special composition buffered media to reduce the effect of osmotic shock (Mortimer D)
- **Results**: Better yields, but was not usually of major benefit in patient-specific clinical situations.

Percoll - Albumin

ALBUMIN:

- Highly effective in promoting **sperm survival** (and **capacitation**)

- **Should be included in Percoll process**. (if possible, in gradient layers themselves, but certainly in wash medium).

- **Less than 10 mg/ml**: increased expression of the **sticking-to-glass phenomenon**: motility assessments in the absence of "reasonable" albumin concentration less reliable.

Percoll - PVP - Good & Bad

The Good

- Percoll made of PVP-coated silica particles: a mineral

Hence: Very little osmotic activity.

- Dense gradients can be made with little osmotic or viscosity side-effects; a major plus when separating dense cells such as sperm.

And... The Bad

However: Silica particles are known to be a chronic tissue irritant and, will kill sperm DEAD

- Pertoft's PVF coating was such a breakthrough.

Reality

- PVP might be cause for some concern,
- But no dramatic problems from thousands of ICSI injections performed using it!

Percoll

What does all this mean?

Basically, if one of the less-than-ideal Percoll methods were used, the sperm may well have poorer functional potential than direct swim-up from liquefied semen, which remains a highly effective sperm prep method for men with more-or-less normal sperm.

What about Pharmacia's withdrawal of Percoll?

Not profitable:

- ART users represent a small part of Percoll market - Pharmacia not interested in increasing the cost of product by making it to standards certified for clinical use

Problem:

- Some batches of Percoll have high **endotoxin** levels
- Contaminated batches are then used for less critical applications such as research and diagnostic sperm preps.

What about Pharmacia's withdrawal of Percoll?

Nowadays

Pharmacia Biotech' s statement:

- Percoll is manufactured for research purposes only and is not allowed for isolation of cells for clinical purposes.
- Pharmacia asks the end-users a signed declaration that Percoll is not used in human IVF or ICSI

Alternatives to Percoll

- When Percoll was pulled from use in the human infertility market other substitutes quickly appeared.

- Most of these new gradient methods involve the use of silica coated with hydrophilic silane (as opposed to PVP in Percoll).
- Of course most of these "new" gradient separation methods are about 3 to 4 times as expensive as Percoll.

- What is interesting is that the manufacturer of silanized silica is Pharmacia.

Alternatives to Percoll

Nycodenz gradients for clinical sperm preps in male factor IVF patients useful, but:

- gradient technique was more difficult to use than Percoll,
- method never really took off

Alternatives to Percoll

Recently, manufacturers (now called Nycomed) have a new product called **OptiPrep**

- a dimeric form of Nycodenz iohexol molecule (called iodixanol) and close to Percoll.

- gradient method is pretty much as the same as for Percoll, but off-the-shelf product is less easy to prepare into gradients: being a small organic molecule it is more osmotically active than sand and formulating gradients is consequently less easy for non-research labs.

Alternatives to Percoll

Ficoll and other carbohydrate-based density gradient materials are really too osmotically active and, when made up to the necessary density for separating sperm, too viscous to be clinically useful

Alternatives to Percoll

PureSperm
(from NidaCon Laboratories AB of Gothenburg Sweden)

Technique:

A simple 80%/40% (v/v) gradient with 1.5 ml layers spun at 300 g for 20 min with recovery of the soft pellet and a single wash at 500 - 600 g for 6 min.

Alternatives to Percoll

"Isolate" (Irvine Scientific)

It is basically a colloidal suspension of silica particles in a HEPES-buffered medium used as a two-gradient system.

- Post-separation sperm samples look very similar to those following Percoll.
- IVF fertilization and embryo quality were equivalent to Percoll.

Alternatives to Percoll

Studies comparing PureSperm, Isolate & Optiprep with Percoll

- The **first two were superior** with Optiprep lagging behind in sperm recovery.
- Puresperm comes more conveniently in larger bottles and is simply diluted with culture medium. Two 1 ml layers work well.
- Optiprep and several others, eg CellSep from the Larex Corporation and the Optiprep/Ficoll combination : bring down many immotile sperm along with the motile ones which the silica based products (Percoll, PureSperm, Isolate) do not. One solution to this problem is to then use a swim up from the collected pellet

Alternatives to Percoll

A new technique: Multi-ZSC

- This method uses a multi-compartment column that allows the entrapment of the various subpopulations of spermatozoa as they swim up.
- The Multi-ZSC, because of its unique design, allows the entrapment of the most fit sperm for the ART's and particularly for ICSI techniques where the selection of male gametes is of utmost importance in determining the outcome of IVF.
- Preliminary results indicate that the recovered spermatozoa from the top compartment of Multi-ZSC are superior to those obtained via traditional swim-up method.

Alternatives to Percoll

- Rather than debating on what triggered this action by Pharmacia, is it not advisable to carry out a systematic scientific study on the toxicity of Percoll?

- Major issue against Percoll is the batch to batch variation in the levels of endotoxin.

  - Basically, a problem of quality control. Cannot each batch be tested prior to its release?

  - In a preliminary study, Jim East reports that the endotoxin levels are undetectable in the sperm suspension obtained after the preparation of Percoll.

Alternatives to Percoll

- Can we condemn a product just because some particular batches are toxic?

- Percoll primarily consists of two substances - polyvinylpyrrolidone (PVP) and silica particles. If one of these is toxic then it is ironic that most of the new alternatives are based on silica particles while PVP is routinely used for immobilization of sperms for ICSI !!

- Why not scientifically tackle a problem rather than desperately searching for alternatives?.

SWIM UP

- Some authors: less than ideal in male factor cases (and increasingly so with more severe cases).
- Others: most suitable method for sperm prep during IUI and most of cases in IVF.
- Purity of the sample with swim up depends on the primary sample motility grade and the total number of the motile sperms.

 - Best uses of using the swim up method is it gives normal, good grade motile, sperms in the yield.

- **Disadvantages:** lower yields but the fact is retrieval of oocytes doesn't exceed 30 in any case and number of sperms required to fertilize in an IVF programme is about 10000 motile sperms of good grade per oocyte

IN SUMMARY

- Majority of labs report excellent success, and fertilization rates (> 80 or 90%) by using a gradient prep of the sperm; or a gradient prep followed by swim-up.

- The more successful labs, if a swim-up is done, will swim-up from semen, not from a washed pellet.

Ultimately, it' s the native semen sample and the embryologist concern who will have to decide which method needs to be adopted.