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Review Article

Sperm processing techniques for Intra-Uterine Insemination

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Abstract

Intrauterine insemination (IUI) remains safe, simple relatively cost effective and a valuable initial treatment option, especially in low resource settings for selected group of patients before embarking to assisted reproduction technology treatment. Assisted reproductive technology (ART) does not include assisted insemination by sperm from either a woman's partner or a donor. Numerous factors are influencing the success rate of IUI. However, sperm processing method is a modifiable factor in an induvial basis to achieve good success rate. Aim of sperm processing methods are to target analogously and effectively filter progressively motile and morphologically normal sperm from the overall sample population resulted an enriched sperm population with higher fertilizing potential. WHO recommended sperm processing methods are usually adopted in Sri Lanka and major categories are Sperm Migration Method, Density Gradient Centrifugation Method and Column Adherence Method. Each methods have advantages and limitations in clinical practice. Therefore, andrologist and fertility specialist need to decide the best possible sperm processing method for a particular couple for optimal success rate of IUI treatment according to the clinical assessment and sperm quality and quantity.

Introduction

Intra-Uterine Insemination (IUI) is an assisted conception technique involving the deposition of processed semen into the uterine cavity around ovulation time (1,2). It is a simple technique performed with minimal infrastructure facilities and fewer risks for subfertile couples (3,4). Women with patent fallopian tubes and infertility due to male-factor, unexplained factors, cervical factors, immunological factors and ejaculatory disorders are usually indicated for this treatment. It has a 10-20% clinical pregnancy rate. However, the technique of IUI, ovulation stimulation protocols, sperm preparation techniques and ultrasound monitoring of follicular development have led to promising success rates (5).

Physiological changes of the sperm prior to natural fertilization

During coitus, the ejaculated coagulum becomes deposited around the external orifice of the cervix and the posterior fornix of the vagina. Freshly ejaculated, this coagulated semen protects sperm from the acidic vaginal environment and facilitates sperm transport through the cervix and fallopian tubes. As the coagulum begins to liquefy, otherwise trapped sperm are released into the surrounding environment. Most motile sperm with normal forms will then rapidly penetrate and migrate into the cervical mucus. A tiny fraction of these sperm might eventually reach the oocyte within the fertilization area. Those sperm remaining behind within the vagina are inactivated and summarily destroyed, likely due to the relatively high acidity of the vaginal confines.

Interestingly healthy sperm, although fully formed at the moment of ejaculation, are not yet able to fertilize an egg. Prior to making contact with the oocyte, sperm must undergo further physiological maturation possible only within the female reproductive tract, called "capacitation" (6,8). Such capacitation is inhibited outside the female in order to conserve sperm fertilization capacity. Specifically, decapacitating factors present within the seminal plasma itself prevent sperm from undergoing spontaneous and independent capacitation reactions. Once semen has been ejaculated into the vagina, several factors, including cervical mucus, will facilitate the capacitation process occurring within the reproductive tract. Also, the periovulatory mucus acts as a barrier against leucocytes, prostaglandins, and various infectious agents present in the seminal fluid. It allows only progressively motile sperm to penetrate and migrate through the cervix with normal shape and size.

Sperm processing techniques

Sperm processing techniques have been designed to duplicate these natural physiological functions of the female reproductive system. Logically, such techniques should produce the most optimal results. It mimics the

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sperm separation abilities of periovulatory mucus that can favorably influence the reproductive outcome. These methods analogously and effectively filter progressively motile and morphologically normal sperm from the overall sample population. The resultant sample contains an enriched sperm population with higher fertilizing potential.

Central to this process is separating decapacitation factors and contaminants from the seminal plasma, such as cellular debris, gelatinous pieces, epithelial cells, bacteria, leukocytes, and erythrocytes. The goal should be thorough filtering of motile sperm and the recovery of most, if not all, viable spermatozoa. Such separation procedures should be reliable, repeatable and relatively simple to perform. In Sri Lanka, WHO recommendations for sperm processing for IUI are widely adopted in clinical practice.

These separation procedures can be broadly categorized into three major groups:

- Sperm Migration Method
- Density Gradient Centrifugation Method
- Column Adherence Method

Sperm Migration Method

Motile sperm are selected based on their natural ability to migrate into a defined medium. In the sperm swim-up procedure, a low-viscosity medium is layered over the semen. The motile sperm are allowed to migrate up against gravity, leaving all other no dynamic factors within the sample behind (9).

It is also possible to recover motile sperm by allowing them to migrate down with the help of gravity into a higher viscosity composed of bovine serum albumin. This procedure has been claimed to yield a higher concentration of Y chromosome–bearing sperm (Ericsson, Langevin, and Nishino 1973). However, it is not recommended for routine use. All the procedures mentioned above allow motile sperm to swim up or down into a gradient, separating them from the nonmotile fraction and ejaculatory cellular debris left behind. They will generally yield a population of motile sperm. However, it should be noted that although sperm motility is a prerequisite for conventional IVF, not all motile sperm are fertile.

Density Gradient Centrifugation Method

Sperm are selected based on their motility, size and density differential as they are centrifuged through a continuous or discontinuous density gradient of either colloidal or salinized colloidal silica (11). Compared with sperm swim-up or swim-down procedures, density-gradient centrifugation (DGC) procedures yield a higher concentration of motile sperm(12) and are therefore considered to be industry-standard procedures for processing semen for IUI. However, the DGC method is not recommended for extremely low sperm content semen samples, highly viscous semen samples, or samples containing a large percentage of cellular debris.

Discontinuous DGC techniques were used extensively before IUI and IVF to separate motile spermatozoa from immotile spermatozoa and other cells and eliminate decapacitation factors, prostaglandins and reactive oxygen species (ROS) (13). Generally, a motile mature spermatozoon has a higher density than an immotile or immature spermatozoon (14). After centrifugation, leukocytes and cell debris are concentrated in the seminal plasma and upper layer interface. Morphologically abnormal spermatozoa collect in the interface between the upper and lower layer, and motile and mature spermatozoa form a pellet at the bottom of the tube (15).

DGC effectively separates motile from immotile spermatozoa and yields a low concentration of ROS. The reduced ROS in the lower layer, relative to the unwashed samples, strongly suggests that the treatment of semen by DGC does not expose motile sperm to oxidative stress (11).

Column Adherence Method

Sperm are selected based on the fundamental concept that nonviable sperm are ‘’sticky,’’ and, therefore, more likely to adhere to the silica (glass) wool column than otherwise motile and functionally intact spermatozoa (16,17). Compared with the swim-up sperm and density-gradient procedures, filtration procedures yield higher concentrations of sperm, especially in cases of viscous and/or asthenozoospermic and oligozoospermic ejaculates (18). The filtered sperm also has a higher percentage of intact acrosomes (19). and chromatin integrity (20). In addition, filtered sperm yielded significantly higher results in the zona-free hamster oocyte sperm penetration assay (SPA) (21). They bound more in the zona-binding assay (22). Finally, filtered sperm resulted in a higher percentage of oocytes fertilized during in vitro fertilization than sperm recovered from the swim-up procedure (23). Sperm recovered after filtration could be successfully used for IUI.

Processing Retrograde Flow of Semen

Semen is directed into the urinary bladder during ejaculation, usually with aspermia. It is confirmed by examining a sample of post-ejaculatory urine for the presence of spermatozoa. Due to its acidity and other factors, urine is naturally detrimental to sperm quality. The first step toward successfully procuring viable sperm from urine involves chemically altering the osmolality and acidity of the bladder urine.

Since urine above 40% volume-to-volume concentration is reported deleterious to sperm function (regardless of whether urine pH and osmolality are first modified), the clinician should collect urine aliquots in a buffered physiologic solution or medium (24, 25). Collect the first aliquot of 5 to 10 ml of voided urine in 10ml of sperm processing media and immediately centrifuge at 500 x g for 5 minutes and discard the supernatant. Resuspend the resultant sperm pellet in a 5 ml medium and centrifuge at 500 x g for 5 minutes, and discard the supernatant. If sperm processing is deemed necessary, resuspend the resultant sperm pellet in the medium and proceed accordingly as described above.

Some studies have suggested that alkalinization of the urine by ingestion of sodium bicarbonate 24 h before and one hour prior to ejaculation, would increase the sperm quality in the urine. However, this method may disturb the patient's acid-base balance in the body (26, 28).

Converting the retrograde flow of semen to antegrade ejaculation may be attempted through various medical treatments. Sudafed 60 mg or Imipramine 25mg 4 times per day for 7-10 days prior to scheduled semen analysis (including tablet morning of collection). Collect and evaluate both antegrade and retrograde flow of semen. Compare results from both treatments. Choose most efficient pharmacological agent (3).

Conclusion

IUI is a safe, simple and relatively inexpensive fertility treatment for subfertility couples in low- and middle-income countries like Sri Lanka. The sperm processing technique plays a pivotal role in IUI treatment and its success. Although sperm processing procedures attempt to mimic the innate capabilities of the female reproductive system, in reality, these laboratory techniques are able, at best, to select a more suitable sperm population based solely on particular sperm characteristics.

Sperm Migration Methods all rely on progressive sperm motility, whereas Density Gradient Centrifugation Methods rely on shape, size and sperm density. The Sperm Adherence Method, on the other hand, mainly selects and removes sperm with broken or non-functional sperm membranes. Essentially, no laboratory technique developed thus far indeed and comprehensively mimics the periovulatory mucus. The latter two methods can concentrate most viable sperm into volumes sufficient for IUI but in proportions greater than those attainable by the periovulatory mucus itself.

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