—Mini Review—

Human Sperm Processing in Assisted Reproduction Technology

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Abstract: *In ART, once ovum is successfully yielded, embryologists have to progress the treatments as arranged regardless of the quality of semen. Sperm qualities are, therefore, the most variable factor in each case. Embryologist is certainly well informed about physiology and genetics of ovum and embryo, it is also essential to study how to evaluate and prepare the sperm according to their physiology.*

Key words: *Human sperm, ART, DNA, Motility*

Progress in assisted reproduction technology (ART) has intended to make shortened the distance between the male and female gametes prior to fertilization. Intrauterine insemination (IUI) omits the passage through the cervix artificially, and IVF-ET facilitates the fertilization by incubating the oocyte and the sperm, *in vitro*. The ultimate method, intra-cytoplasmic sperm injection (ICSI), the distance between the two types of gametes becomes 0 by means of artificial injection. The aim of sperm preparation in ART is to deputize for some of the events which occur in the female genital tract.

Spermatogenesis

Sperm is the end product of the process of gametogenesis in the male, occurring within the seminiferous tubules of the testes. This involves a series of meiotic divisions of spermatogonial stem cells, two meiotic divisions by spermatocytes, extensive morphological remodeling of the spermatid during spermatogenesis, and the release of the free cell into the lumen of the seminiferous tubule by spermiation. It is an interesting paradox that the process of spermatogenesis produces a cell that is highly differentiated in structure and function, while at the same time is developmentally totipotent, being able to combine with an egg and thereby begin the process that give rise to a new individual.

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Ejaculate

Many millions of sperm are produced daily in the testes, these are stored in the epididymis and released at regular intervals (ejaculation). The ejaculate once coagulates and liquefies at room temperature after about 10–30 min due to protein splitting enzymes from the prostate. Although the semen contains a variety of components, only the sperm with progressive motility start to penetrate into the cervical mucus and finally a small part of them reach the ampulla of the uterine tube. All the other components such as immotile sperm, bacteria, leucocytes, and the seminal plasma remain in the vagina.

Sperm DNA

Mammalian sperm has two main components, the head and tail. The head consists of the acrosome, the nucleus and a small amount of cyto-skeltal structures and cytoplasm. The acrosome is a large secretory granule that closely surrounds and overlies the anterior end of the nucleus. The sperm nucleus is haploid, containing only one member of each chromosome pair, and chromatin becomes highly condensed during the latter part of spermatogenesis. The volume of the sperm nucleus is less than that of somatic cells, and its chromatin is highly condensed. The sperm nucleus is unique, both in the amount of DNA present and in the composition of its nucleoproteins. The major nucleoproteins associated with sperm DNA are protamines. These are relatively small (27–65 amino acids) highly basic proteins rich in arginine and cysteine. The highly condensed DNA-protamine

complex is stabilized by disulfide bonds between protamines.

Programmed cell death is recognized as an essential event in morphogensis as well as normal turnover of cells [1], and it is known as apoptosis, which distinguishes it from necrosis or pathologic cell death [2]. Apoptosis plays important roles in germ cell loss [3]. It is the dominant process during spermatogenesis and is regulated by p53, p21, caspase, bcl2, and Fas expression levels, with many alternative path ways [4]. It is commonly recognized that human ejaculate contains heterogeneous sperm populations, which possess a variety of abnormalities at nuclear, cytoskeletal and organelle levels. To date, numerous authors have reported the existence of sperm with DNA damage, and the rate was increased in severe oligo-asthenozoospermia [5, 6].

Quality assurance of human sperm provided for ICSI

Since ICSI has diminished the quantitative limitation of sperm required for insemination as minimum as possible, it is accepted that most male infertilities can be overcome, except azoospermia. In ICSI, the injection of sperm is regarded as the transplantation of male chromosomes into the oocyte. As described above, the ejaculate has a heterogeneous sperm population. The selection of sperm in ICSI is, however, merely based on motility and gross morphology under a low magnification microscope. There is no proven method for sorting sperm by DNA integrity. The quality assurance of the sperm for the injection, especially the integrity of the DNA structure, is a minimum premise for the clinical application of ICSI in severe male infertility.

To resolve the above issue, three innovative technologies are necessary. First, we need to develop some methods to measure nuclear damage at multiple levels such as chromosome, gene and DNA structures in individual human sperm with high detection sensitivity. Second, the sperm, which possess DNA damage prior to ejaculation, have to be eliminated by means of the *in vitro* processing, and post-ejaculate DNA degradations have to bereduced to the minimum.

Significance of human sperm preparation in ART

During migration in the female genital tract, the sperm is selected and undergoes various physiological changes to achieve fertilization. The aim of sperm selection in ART is to synthesize these events by the *in vitro* processing. The ejaculate is polluted with bacteria during passage through the urethral orifice. Bacteria should be sterilized by the addition of antibiotics to the suspension or separated physically according to the differences in their apparent densities. After centrifugation, the sediment (sperm) is often recovered by aspirating the supernatant with a Pasteur pipette. In this way, a part of the supernatant adhering to inner surface of the tube descends, and the sediment is re-polluted with bacteria.

The ejaculate includes not only the sperm but also a variety of debris such as fine urethral calculus, mucinous gel, fibers of under wear, etc. Bacterial colonies are often observed on the surface of debris. The density gradient centrifugation technique is capable of separating free bacteria in the ejaculate, whereas those adhering to the debris are recovered in the sediment. After liquefaction, a small number of sperm with progressive motility start to penetrate into the cervical mucus, and all other components remain in the vagina.

To date, various procedures for sperm preparation have been reported [7–10]. Figures 1 and 2 show sperm preparation procedure to exclude debris in the ejaculate and for selecting the sperm with progressive motility. Prior to centrifugation, the debris has to be excluded by means of unit gravity sedimentation and subsequent filtration (ART filter, Nipro, Japan). The resulting suspension is condensed by the cushion method. Then the concentrated fraction is loaded on 5.0 ml of 90% Percoll in a separable fine neck tube (SFNT, Nipro, Japan), which is squeezed at the bottom to make the volume of sediment as small as possible. The linear density gradient is created by slow rotation, and centrifuged at 400x g for 30 min. To recover the sperm precipitated in the bottom of the tube, the top of the tube is plugged with a rubber cap, and the middle of the squeezed bottom is snapped off to avoid recontamination of the sediment by the seminal plasma and bacteria.

The motile sperm are separated by the modified swim-up method. The motile sperm are allowed to swim-up at 37°C, and the upper layer containing swum-up sperm is collected.

Observation of DNA fragmentation in human sperm

To observe DNA fragmentation in individual sperm, we employ single cell gel electrophoresis. The sperm is

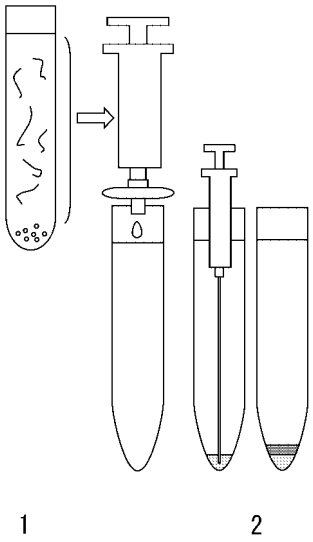

Fig. 1. Semen pretreatment prior to centrifugation. 1. Dilute semen and allow to precipitate debris. 2. Filtrate the suspension, introduce 0.1 ml Percoll to the bottom of the tube, then centrifuge (cushion method), then centrifuge.

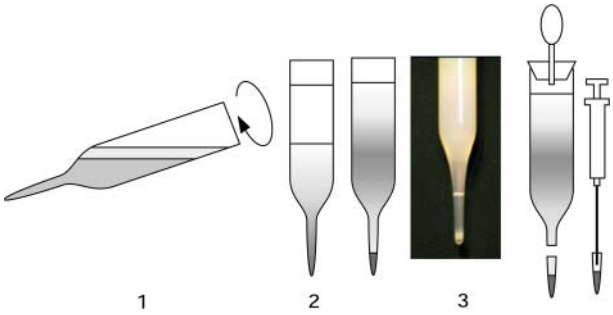

Fig. 2. Sperm fractionation in the continuous density gradient of Percoll. 1. Isotonic 90% Percoll (5.0 ml) was placed in a centrifuge tube; 1.0 ml of Hanks solution is loaded and a linear density gradient is formed by slow rotation. 2. The sperm re-suspension is layered, then centrifuged at 400 xg for 30 min. 3. The end of the test tube is cut off to recover the sediment. 4. The sperm suspension is introduced into the bottom of the culture medium to swim up. 5. Recover of swum-up sperm.

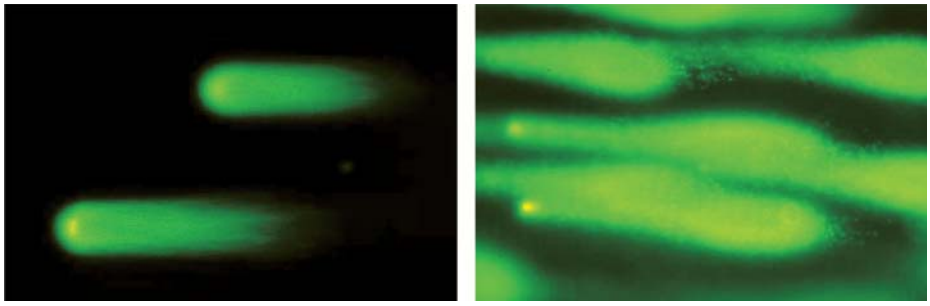

Fig. 3. Electrophoretogram of human sperm. Left: sperm with DNA integrity, Right: sperm with fragmentated DNA.

suspended in melted agarose, and a thin layer is formed on the surface derivatized glass slide. The disseminated cells are lysed with some detergent and proteinase (trypsin). Electrophoresis is performed at pH 8.4 for 10 min (2.0 V/cm voltage gradient). Figure 3 shows the electrophoretogram of human sperm. Intact sperm gives continuous DNA fibers from the origin (left photo). On the other hand, sperm with DNA fragmentation gave elongated granular particles.

Conclusion

The essence of ART is transplantation of the male chromosomes into the oocyte. Quality assurance of the sperm chromosome is, therefore, essential in not only ICSI, but also the other insemination techniques. Microscopic measurements of sperm concentration, motility and morphology may describe some aspects of the function, whereas the predictive value of these measurements is limited, even Ithough some progress has been made in recent years by the developments of the computer assisted sperm motility analyzer. In future, some molecular biological examinations of the sperm function, especially DNA integrity may play important role for the infertile therapy.

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