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REVIEW

Sperm capacitation: effect of assisted reproductive technology

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INTRODUCTION

The past several decades witnessed great advances in the Andrology Sciences in humans. This certainly increased our knowledge in understanding the process of spermatogenesis, capacitation, and fertilization. Much of this work has been acquired from animal experimentation (1, 2, 3). However, the introduction of assisted reproductive technology improved our understanding of the process of fertilization in humans and increased the research in this field to improve the success of the treatment of the infertile couple (4, 5).

The process of spermatogenesis depends on an intact hypothalamic-pituitary testicular axis and an intact internal hormonal milieu. Follicle-stimulating hormone stimulates the process of spermatogenesis. Leutinizing hormone stimulates the Leydig cells to secrete testosterone which is important for the maturation of the sperm (6, 7). It takes about 70 days for the spermatogenesis cycle to be completed and the sperm to be delivered to the epididymis for full maturation. In addition to the spermatozoa the ejaculate contains secretions from the prostate, and seminal vesicles. Once ejaculation occurs, a coagulum is formed, and this will liquify within 20 minutes by the prostatic enzymes present in the ejaculate.

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Sperm capacitation

Sperm capacitation has been described as a process in which the sperm in the ejaculate undergoes certain changes in the acrosomal part to allow this sperm to penetrate the cumulus and the zona pellucida to reach the cytoplasm of the oocyte. This process was described in the rabbit and rat animal models to take up to 10 hours or so to complete. This process of capacitation of the sperm takes place in the genital tract (8, 9).

It is believed in humans that the process of capacitation takes place in the cervical mucous. Following ejaculation in the vagina many sperm will move into the cervical mucous and the rest of the sperm will die in the vagina because of the acidic pH. The cervical mucous has to be suitable for this process. Under the effect of estrogen the cervical mucous is increased in amount becomes

clearer, and more fluid; thus allowing the sperm to move in it. The cervical mucous acts as a reservoir from which the sperm will move toward the uterine cavity and fallopian tubes. Sperm was found in the cervical mucous within 90 seconds after ejaculation (10, 11), and in the peritoneal cavity within half an hour after ejaculation. This demonstrates how the sperm moves very quickly provided that the mucous and other parts of the uterine cavity and fallopian tubes fluid are suitable for this process (12).

The sperm, after leaving the ejaculate and moving in the cervical mucous, gets rid of all the covering seminal fluid. This seminal fluid probably is referred to as the decapacitation factor. So, the sperm will be capacitated after passing through the cervical mucous without the seminal plasma. The sperm acquires increased motility to facilitate penetration of the cumulus oophorus. The acrosome will then be ready to undergo the necessary reaction when it meets with the oocyte (13). This process probably exposes the receptors that will be able to attach to the oocyte. The oocyte is surrounded by the zona pellucida which is composed of three glycoproteins, called ZP1, ZP2 and ZP3. The process of fertilization takes place when the capacitated acrosome-intact sperm binds to ZP3. The binding of ZP3 to the sperm induces the acrosome reaction. The acrosome-reacted sperm now appears to be associated with ZP2 and begins to release acrosomal enzymes. The outer acrosomal membrane will formulate vesicles that will lead to the secretion of several enzymes including acrosin and hyalurinidase, a trypsin-like enzyme to hydrolyze the zona pellucida glycoproteins and facilitate the penetration movement of the sperm through the zona pellucida. Following that, the inner part of the acrosome membrane that is covering the nucleus of the sperm head will fuse with the nucleus of the oocyte and the two pro nuclei are formed indicating that fertilization is successful and this will then lead to division of the cell and formation of the embryo. The sperm once inside the zona pellucida binds to oolemma and induces the cortical reaction resulting in release of oocyte cortical granules. One of the enzymes released by the cortical granules cleaves ZP3, forming a glycoprotein now called ZP3-F, which is no longer capable of binding sperm. This also is called the “zona reaction” and is important in the prevention of polyspermia (15, 16).

Several factors may interfere with this capacitation process including coating of the sperm with sperm antibodies and the effect of smoking and drugs on the DNA system of the sperm, thus making it unable to undergo this process, and therefore, leading to infertility.

Sperm Antibody

The immune system may recognize the protein of the sperm as foreign; and therefore, antibodies will be secreted towards the sperm. Approximately 8-10% of sexually active men or women develop antibodies to sperm. 50-60% of men with history of trauma, vasectomy, infection and mumps have sperm antibodies. The blood testis barrier and male genital tract effectively prevent the development of antisperm antibodies in most individuals. But such factors as trauma, vasectomy and infection compromise this barrier and permit the development of antisperm antibodies. In men this is usually common after sperm ligation because of the buildup of the sperm in the epididymis and some of the sperm may gain access to the lymphatic system or the vascular system as a result of the pressure built up in the epididymis. This is why sometimes after vas anastomosis, the sperm ejaculated is found to have poor motility and agglutination effect. Study of sperm antibodies on the sperm was found to be very high and that is one of the causes of failure of vas anastomosis in achieving pregnancy rates (17). Antibodies have also been found in men as a result of infection of the testis or epididymis, also as a result of schistosomiasis in some parts of the world where schistosoma is common. Sperm antibodies have also been found in women in the cervical mucous as a result of activation of the immune system towards that sperm (18, 19).

There are two types of sperm antibodies; sperm agglutinating antibodies and usually this is present on the head of the sperm and will lead to agglutination and clumping of the sperm. Sperm immobilizing antibodies usually attach themselves to the tail of the sperm and lead to poor motility. Both types of sperm antibodies are associated with

infertility, due to poor motility and sperm penetration of cervical mucus (20).

Sperm washing technology that is used in assisted reproduction is usually not successful in removing the sperm antibodies from the sperm. This is because the sperm antibodies are usually binding very tightly to the cell membrane of the sperm and probably of the covalent type that cannot be separated successfully by sperm wash. This will lead to inhibition of sperm-zona pellucida binding (21).

Several investigators used steroid therapy for men who demonstrated the presence of sperm antibody. The steroid therapy inhibits the immune system and the sperm antibody concentration declines and the sperm then can be used for insemination (22, 23). However, if there is a contra-indication, of course, to steroid therapy then the best approach to the treatment of these patients is to do in vitro fertilization and intracytoplasmic sperm injection (ICSI).

Varicocele

Several investigators in the past correlated the presence of varicocele with abnormal sperm picture, whether it is low sperm count or abnormal morphology or poor motility (24). As a result of that, varicocelectomy has been a procedure that was used repeatedly to treat varicocele. However, the various reported studies do not have very good controls. Therefore, it is difficult to make any scientific judgment regarding the role of varicocele on sperm production. It was stated that varicocele increase the heat around the testis and also may lead to reflux of steroids and catecholamines from the left renal vein, thus, decreasing the oxygen tension leading to stress effect on the testis (25,26). These views have not been recently supported and there are many men with varicocele who have fathered children. Reproductive endocrinologists really do not have much interest in varicocelectomy. Furthermore, treatment using gonadotropins and other fertility medications has not been very effective in improving the sperm picture. For this reason, the best approach to the abnormal sperm pictures in such patients is to go for assisted reproductive technology. Intrauterine insemination is used usually for about three cycles and if this is not successful, then in vitro fertilization using ICSI will be the proper management in such patients.

Abnormalities in the sperm picture whether it is low count, abnormal morphology or poor motility might be due to serious problems related to the DNA of the sperm (27). Therefore, it is probably wise and indicated that in these cases with abnormal sperm picture, the clinician should think of ordering chromosomal studies and/or DNA studies. The changes in the DNA could be related to smoking, drugs, and other environmental factors.

Intracytoplasmic Sperm Injection

Intracytoplasmic sperm injection, known as ICSI, has been introduced in the early 1990's for treatment of male infertility. However, along the years, this technology has been used also for other infertility conditions including failure of conventional in vitro fertilization technology, women above the age of 35 years due to hardening of the zona pellucida and in the presence of sperm antibody cases. In this technology a sperm is picked up in a micro pipette and injected directly through the zona pellucida into the cytoplasm of the mature oocyte (28). The fertilization rate is usually very high in these cases. There have been various studies comparing the pregnancy rates between conventional IVF and ICSI. A Cochrane review study showed that there is a significantly higher fertilization rate in the IVF group but no difference in pregnancy, miscarriage or live birth rate. There are several reports dealing with the rate of birth defects after assisted reproductive technology. It has been reported that the incidence of fetal anomalies is higher after IVF and ICSI compared to controls. There is no difference in the rate of these anomalies between IVF and ICSI pregnancies (29).

It is known that men with oligospermia may have either chromosomal abnormalities or mutation in the cystic fibrosis factor. Certainly, if ICSI is the method of fertilization, the sperm that carries these anomalies could be the fertilizing sperm. For this reason, these men with sperm abnormalities should have chromosomal studies (30). They should also be tested for DNA

fragmentation, especially if they are smokers or have varicocele. This testing is certainly suggested and it should not be taken against the treatment of the couple.

Intrauterine Insemination

Intrauterine insemination was introduced in the 1980's as part of the treatment of infertile couples due to several problems including male factor infertility, cervical mucous factor, sperm antibody, and unexplained infertility. In this technology the sperm is collected in a sterile container in the Andrology Lab area. The sperm is then washed and concentrated in 1-3 ml of the culture medium and is used for intrauterine insemination. The value of this technology is to have a high concentration of sperm bypassing the cervical mucous factor (31). This sperm deposited in the uterus will be in close proximity of the tubal openings and therefore will have quicker accessibility to the oocyte. Certainly, washing the sperm increases the capacitation and helps the fertilization rate. The pregnancy rate in these situations is between 30 and 40%.

The main objective of sperm preparation is to isolate the maximum number of motile sperm without causing any damage, eliminate dead sperm, amorphous cells, leukocytes, and separate seminal plasma from the ejaculate. Various methods of sperm wash preparation have been used including swim up, swim down, glass wool filtration, and density gradient centrifugation (32). It has been shown that the swim up and swim down technology and the glass wool filtration leads to marked reduction of the available sperm. Therefore, the density gradient centrifugation is the best acceptable technology because it yields more motile sperm and provides sperm with better DNA quality (33, 34, 35). Several reports in the literature suggest that the density gradient centrifugation gives a higher pregnancy rate than the old technology depending on the swim up and swim down technology (36, 37). The sperm washing preparations eliminates decapacitation factors by separating sperm from the seminal plasma and render the sperm more capacitated and helps the acrosomal reaction. It is suggested that a minimum of three cycles of intrauterine insemination should be performed before considering in vitro fertilization. Intrauterine insemination using washed sperm could be used in natural or stimulated cycles. Certainly, using controlled ovarian stimulation will help improve the pregnancy rates in these patients.

CONCLUSION

Assisted reproductive technology allowed embryologists and andrologists to improve on the capacitation process. The result of this technology is to separate the morphologically normal sperm, improve the motility, and remove all decapacitation factors. The capacitated sperm has a better chance for fertilization either by the use of intrauterine insemination or the various IVF technologies. Fertilization can also occur by the technology of ICSI in the presence of sperm coated with sperm antibodies. Reproductive endocrinologists and infertility specialists must consider chromosomal karyotyping for men with abnormal sperm pictures especially those with poor sperm morphology. DNA studies of such sperm may reveal fragmentations. During pregnancy, genetic counseling studies should be considered to detect any fetal abnormalities.

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