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Nurture vs Nature: How Can We Optimize Sperm Quality?

Andrology Lab Corner*

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The number and quality of sperm produced by the testis is determined to a great extent by the interaction of three factors: 1) the genetic make-up of the spermatogonia; 2) normal Sertoli and Leydig cell function; and 3) the interaction of Sertoli, Leydig, and germ cells with endogenous factors and the environment. Even if the genetic endowment of Sertoli and germ cells were to be optimal, sperm quality and number can be greatly compromised by environmental factors. The importance of germ cell genetics in sperm production and quality is exemplified by the sperm produced by Holstein bulls, compared with their predecessor bulls in the wild. Through more than 100 years of successful breeding, those genes that optimize spermatogenesis and spermiogenesis have been selected in the testis of these bulls, but those genes that have a negative impact on these processes have been deselected from their genome. However, if we were to place these so-called superbreeders in a hostile environment, those attributes that make them such a wonder of nature could be greatly compromised (Mathevon et al, 1998). On the other extreme of the spectrum are infertile human males with severely compromised testicular function. In these males, sperm production and quality is decreased because of constitutive genetic deficiencies that affect both primary genes and gene modifiers involved in sperm production. In these males, sperm nurturing becomes limiting in terms of maintaining adequate sperm production and quality.

In this review, we describe two main strategies directed to optimize or ''nurture'' sperm production and quality: preproduction nurturing directed to optimize sperm quality before ejaculation and postproduction nurturing directed to preserve and optimize sperm quality and function after ejaculation.

This article does not attempt to provide a comprehensive review of all the interventions currently used to treat male infertility, but rather it is intended to provide some examples of interventions that can be taken to optimize sperm production in the fertile and infertile male (preproduction nurturing) as well as reproductive techniques used to help bypass defects in sperm function that would probably lead to infertility of the couple under unassisted conditions (postproduction nurturing).

Preproduction Nurturing

A number of environmental factors including high temperature, proinflammatory factors, social habits, drugs, radiation therapy, and xenobiotics have been shown to have a negative impact on testicular function. Perhaps the best example of preproduction nurturing would be simple intervention(s) directed to minimize the impact of exposure to these factors and that might also contribute to improve sperm production and quality. On the other hand, a number of nutraceuticals have been advocated as a way of potentiating sperm production and quality in the subfertile male. In this section, environmental factors and nutraceuticals shown to impact on sperm production and quality are described.

High Temperature

The importance of scrotal temperature in the regulation of sperm production and quality has long been recognized. Variations in testicular temperature have been proposed as a possible determinant of sperm quality (Bedford, 1991; Spira, 1991; Mieusset and Bujan, 1995; Setchell, 1998). Several studies have found a higher scrotal temperature in infertile men, compared with fertile controls, regardless of the cause of impaired fertility, eg, varicocele (Mieusset et al, 1987; Zorgniotti and Sealfon, 1988; Mieusset, 1991). Likewise, in population-based studies, a negative association between scrotal temperature and sperm concentration has been demonstrated (Hjollund et al, 2000). In a retrospective survey of 402 fertile French couples, it was found that it took longer to conceive a child if the man was exposed to heat, eg, baker, welder, than if he was not (Thonneau et al, 1996).

The reason for the variation in scrotal temperature under normal conditions is not well understood, although

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determinants could be of genetic nature. In a recent study, a correlation in median scrotal temperature has been found among monozygotic twins but not in dizygotic twins and a single-born brother (Hjollund et al, 2002). The results of this study suggest a genetic component to the variation in scrotal temperature. A hereditary element in male fecundity may be expressed through scrotal temperature, which constitutes a mechanism independent of those responsible for the development of the sperm-producing epithelium.

An increase in scrotal temperature can be brought about by an increase in environmental temperature, eg, occupational heat exposure in workers of glass and ceramic industry; social habits such as exposure to hot baths, Jacuzzis, saunas, etc or an increase in the endogenous temperature, eg, varicocele, fever. An increase in scrotal temperature not only can result in disruption of the process of spermatogenesis and spermiogenesis but also has been shown to induce sperm DNA fragmentation (Evenson, 2000). Therefore, one way of optimizing sperm quality would be to maintain scrotal temperature at physiological levels by avoiding occupational exposure to heat, treating episodes of fever with antipyretics, or performing varicocelectomy in cases of varicocele, eg, grades II or III, especially in adolescents in whom the presence of a varicocele could significantly compromise their long-term testicular function (Romeo et al, 2003).

Proinflammatory Factors

Chronic inflammatory disease has been shown to affect male reproductive function and fertility. Relevant inflammatory diseases include general and chronic infectious diseases as well as localized acute or chronic infections of the male genitourinary tract. Male accessory gland infections account for almost 15% of all cases of male infertility seen in infertility clinics, but fertility usually is not a clinical objective among patients with acute systemic infections such as Gram-negative sepsis (Hales et al, 1999). Infections of the male accessory glands frequently are associated with increased white blood cell count in semen and elevated levels of proinflammatory cytokines in semen and the testis. There is an increasing body of evidence that demonstrates the importance of cytokines and chemokines in the regulation of testicular and glandular function during pathophysiological states as well as under normal physiological conditions when cytokines act as growth and differentiation factors. Cytokines have been shown to affect Sertoli cell function and alterations in cytokine levels in the testis could affect spermatogenesis (Cohen and Pollard, 1995). Montag et al (1999) reported that use of anti-inflammatory therapy in a male with nonobstructive azoospermia and leukocytospermia resulted in resumption of sperm production and a significant reduction in leukocyte concentration in the ejaculate. Therefore, prompt diagnosis and treatment of these conditions could protect the testis against the negative effect of these proinflammatory factors.

Social Habits

Alcohol consumption and cigarette smoking have been shown to affect sperm production and quality. Cigarette smoking has been associated with reduced sperm count and motility (Kunzle et al, 2003), increased oxidative stress (Saleh et al, 2002), and oxidative DNA damage (Fraga et al, 1991). Chronic ethanol exposure in peripubertal fathers decreases fecundity, and this may be mediated by testicular oxidative injury, perhaps leading to accelerated germ cell apoptosis (Emanuele et al, 2001). In addition, testes of rats fed an ethanol-containing liquid diet had more testicular DNA fragmentation than mice fed an isocaloric control diet. Ethanol increases the number of apoptotic spermatogonia as well as spermatocytes. Direct intratesticular injections of ethanol enhanced testicular DNA fragmentation, suggesting an increase in apoptosis. Moreover, Fas ligand levels are increased within the testes of rats that were chronically fed ethanol. In vitro, ethanol treatment of cultured Sertoli cells enhanced the production of Fas ligand. In addition, testicular levels of p53 mRNA are increased in rats chronically fed ethanol. All of these observations suggest that ethanol enhances testicular germ cell apoptosis (Zhu et al, 2000).

Therefore, males should be aware of the potentially damaging effects of alcohol consumption on testicular function. Investigation into the interplay of constitutive genes and disease modifier genes in males exposed to alcohol would help to reveal whether some of these males develop or not any testicular pathology.

Drugs

A number of pharmaceutical and recreational drugs have been shown to affect testicular and sperm function (Nudell et al, 2002). Some of these include alkylating agents, cocaine, marijuana, calcium channel blockers, cimetidine, colchicine, cyclosporine, erythromycin, gentamicin, neomycin, nitrofurantoin, spironolactone, sulfasalazine, glucocorticoids, and tetracyclines. In a thorough fertility evaluation of the male, the physician should determine what medication the patient is taking as well as recreational drug use (Nudell et al, 2002). Discontinuing the offending agent(s) can reverse most of the induced adverse effects. However, in some instances, such as chemotherapeutic regimens, the medications cannot be discontinued, and pretherapy sperm cryopreservation remains essential (Sweet et al, 1996). Treatment with cytotoxic chemotherapy is associated with significant gonadal damage in men. The likelihood of gonadal failure following cytotoxic chemotherapy is dependent on the type of drug and dose. At present, sperm banking is the

best method to ensure preservation of paternity, although hormonal manipulation to enhance recovery of spermatogenesis and cryopreservation of testicular germ cells are possibilities for the future. Patients with testicular cancer should be informed of the side effects from chemotherapy on testicular function and offered the option of sperm banking of ejaculated sperm (nonazoospermic patients) or testicular sperm (azoospermic patients) (Chan et al, 2001; Schrader et al, 2003) prior to the initiation of the therapy.

Radiation Therapy

Ionizing radiation has been shown to affect testicular function and sperm production. Rowley et al (1974) showed that direct exposure to low-dose radiation (0.15 Gy) causes a significant reduction in sperm count, with temporary azoospermia occurring after exposure to doses of 0.3 Gy.

Radioiodine treatment for thyroid cancer may result in transient impairment of gonadal function (Hyer et al, 2002). The radiation dose absorbed by the testis after a single ablative dose of radioiodine is well below that associated with permanent damage to germinal epithelium and the risk of infertility in these patients is minimal. Patients requiring multiple administrations for persistent or metastatic thyroid cancer may be at greater risk of gonadal damage (Hyer et al, 2002).

Therefore, patients exposed to radiation therapy should be informed of the potential effects of this therapy on testicular function and offered the option of semen cryopreservation prior to the initiation of the therapy, particularly in patients requiring multiple radiotherapy sessions.

Xenobiotics

Xenobiotics and, in particular, xenoestrogens have been implicated in the decline of semen quality observed in the last decades. Some of these include pesticides (Tielemans et al, 1999), insecticides (Hauser et al, 2002), polyvinyl chloride plastics, polychlorinated biphenyls (PCBs) (Rozati et al, 2002), phthalate esters (PEs), and 1,2-dibromo-3-chloropropane (Olsen et al 1990; Teitelbaum, 1999). In a recent study, the highest average of PCB and PE were found in urban fish eaters, followed by rural fish eaters, urban vegetarians, and rural vegetarians (Rozati et al, 2002). The total motile sperm counts in infertile men were inversely proportional to their xenoestrogen concentrations and were significantly lower than those in the respective controls. PCBs and PEs may be instrumental in the deterioration of semen quality in infertile men (Dallinga et al, 2002 Rozati et al, 2002).

Reproductive toxicants such as lead and cadmium have been associated with male infertility (Hess, 1998; Benoff et al 2000; Telisman et al, 2000). Occupational exposure to lead has recently been reported to induce sperm DNA fragmentation (Danadevi et al, 2003).

Benoff et al (2000) and subsequently Marmar (2001), have suggested that cadmium can act as a cofactor in the expression of varicocele-associated pathology in infertile males. They also suggested that other cofactors, such as gene modifiers, could also modulate the expression of this pathology. This may explain, at least in part, why studies involving the efficacy of varicocelectomy on sperm production and quality have not yet yielded definite conclusions concerning its potential beneficial effect.

Xenoestrogens have recently been identified as endocrine disruptors that not only may cause the so-called testicular dysgenesis syndrome (Skakkebaek et al, 2001) but also alter meiosis in germ cells at different stages of development. In fact, cumulative exposure to xenoestrogens has been proposed as a mechanism leading to meiotic alterations in human spermatozoa (Del Mazo et al, 1982).

Use of Nutraceuticals

Nutraceuticals have been advocated as a way of potentiating sperm production and quality in the subfertile male. In a recent study, the administration of folic acid and zinc sulfate to subfertile males was shown to result in a significant improvement in sperm concentration compared to placebo. Treatment lasted 25 weeks and the daily dose of folic acid and zinc were 5 mg and 66 mg, respectively. Although the beneficial effect on fertility remains to be established, this finding opens new avenues of future fertility research and treatment (Wong et al, 2002).

Arginine (Pryor et al, 1978), vitamin B_{12} (Sinclair, 2000), methylcobalamin (Moriyama et al, 1987), and ginseng (Salvati et al, 1996) have been used in the treatment of male infertility. However, most of these compounds have marginal effects on sperm production and quality and have not been tested for safety and efficacy in randomized placebo-controlled studies. In fact, ginseng has been shown to have estrogenic activity (Duda et al, 1996) and produce adverse reactions (Hammond and Whitworth, 1981; Dega et al, 1996).

Because ROS overproduction has been associated with defective sperm function (Aitken et al, 1989), infertile patients have been treated with antioxidant compounds including, ascorbic acid (Fraga et al, 1991), vitamin E (Kessopoulou et al, 1995), selenium (Scott et al, 1998), gluthatione (Lenzi et al, 1998), vitamins, selenium, gluthatione, ubiquinol, and carnitine (Moncada et al, 1992; Lenzi et al, 1993; Costa et al, 1994; Sikka et al, 1995; Vitali et al, 1995; Hawkes and Turek, 2001; Vicari and Calogero, 2001; Lenzi et al, 2003). However, the effect of this treatment on sperm quality is still controversial (Ford and Whittington, 1998; Geva et al, 1998; Lenzi et al, 1998; Tarin et al, 1998; Comhaire et al, 1999). In a randomized, placebo-controlled, double-blind study, oral high doses of vitamins C and E to infertile males did not show any statistically significant improvement in semen

parameters (Rolf et al, 1999). However, in this study, patient recruitment was solely based on having a sperm concentration below 50 million/ml.

One of the main reasons studies looking at the efficacy of antioxidant therapy in the treatment of male infertility have not yet been conclusive may be due to inadequate patient selection. Not all infertile males have an increase in oxidative stress in their testis and semen. Therefore, in principle, only those men who have a quantifiable increase in oxidative stress should benefit from antioxidant therapy. Perhaps the best marker to identify these males would be reactive oxygen species (ROS) levels in semen (Agarwal and Saleh, 2002). Another important aspect of antioxidant therapy is whether the antioxidant(s) and dose used in vivo are appropriate. Previous studies have indicated that the combination of vitamins E and C at high doses in vitro results in DNA fragmentation (Donnelly et al, 1999).

Postproduction Nurturing

In this section, the most common procedures used to preserve and optimize sperm quality and function after ejaculation as well as those reproductive techniques used to help bypass defects in sperm function are described.

Sperm Processing

Density Gradient Centrifugation—Because seminal plasma contains factors that inhibit the fertilizing ability of spermatozoa, it is essential that spermatozoa be separated from it quickly and efficiently prior to any attempts at fertilization. In addition, separation of mature sperm from ROS-producing immature sperm and leukocytes during sperm processing is of paramount importance in preventing ROS-induced damage to the sperm membranes and DNA of the mature sperm (Lopes et al, 1998). It has been previously reported that defective spermiogenesis is associated with the release of high ROS-producing immature spermatozoa into the ejaculate (Aitken and Clarkson, 1987). It has recently been reported that sperm subsets in the ejaculate at different stages of maturation and subsequently isolated by a 3-step density gradient produce different levels of ROS (Gil-Guzman et al, 2001). The interface between 50% and 70% ISolate (Irvine Scientific, Santa Ana, Calif) contains mostly immature sperm with proximal cytoplasmic retention. These sperm produce the highest levels of ROS. Further, ROS levels in this fraction were inversely correlated with percent motility in the sample (Gil-Guzman et al, 2001) and directly correlated with DNA damage, as measured by the sperm chromatin structure assay test (Ollero et al, 2001). Recently, Sakkas et al (2000) reported that sperm isolated from semen by density gradient centrifugation showed a significant improvement in nuclear integrity, as assessed by chromomycin A_3 positivity and DNA strand breakage assays. When prepared using the swim-up technique, the spermatozoa recovered showed no significant improvement in nuclear integrity. Therefore, density gradient centrifugation techniques can potentially enrich the sperm population by separating out those with nicked DNA and poorly condensed chromatin.

Swim-Up—Unlike density gradient centrifugation techniques, which rely mainly on the separation of sperm by cell density (precluding, therefore, the migration to the gradient pellet of the low-density immature sperm), swim-up techniques rely solely on the ability of motile sperm to migrate to the upper culture media layer. Therefore, motile, low-density, ROS-producing immature sperm may be harvested from this fraction. In addition, one swim-up technique involves an initial semen washing by centrifugation step, which has since been shown to result in iatrogenic DNA fragmentation (Twigg et al, 1998). Because oxygen radicals have a short lifetime (nano- to microseconds), bringing immature, ROS-producing spermatozoa and/or leukocytes in close proximity to mature spermatozoa places these sperm at high risk of ROS-induced damage.

Centrifugation of sperm suspensions per se is not harmful to sperm unless mechanical damage is induced by high centrifugal forces. What might be really harmful is the cocentrifugation of mature and immature sperm, leukocytes, or both. Therefore, centrifugation of unprocessed semen should be discouraged and the use of swim-up techniques that bypass a semen centrifugation step recommended, such as direct swim-up (WHO, 1999). Although seminal plasma is known to contain antioxidant enzymes, centrifugation of semen has the dual effect of bringing mature and immature sperm in close proximity. In addition, it will significantly reduce sperm exposure to seminal plasma that, for the most part, will be in the supernatant, leaving a relatively dehydrated pellet devoid of antioxidant enzyme protection. However, these limitations may not apply to semen samples that are devoid of leukocytes or contain relatively low levels of ROS-producing immature sperm. Therefore, damage can be minimized by 1) using the World Health Organization-advocated direct swim-up; 2) using density gradient centrifugation (in which the pellet obtained is comprised, for the most part, of mature sperm); 3) diluting semen samples of high sperm concentration; and 4) use of testicular sperm obtained by testicular sperm extraction instead of epididymal sperm in assisted reproductive technology. Concerning the latter, it has been shown that DNA fragmentation is significantly higher in epididymal compared with testicular sperm (Steele et al, 1999).

In Vitro Treatment With Pentoxifylline and 2-Deoxyadenosine

Previous studies have shown that sperm treatment with pentoxifylline increases the curvilinear velocity, path velocity, straight-line velocity, lateral head displacement, beat cross-frequency, and sperm hyperactivation in both normozoospermic and asthenozoospermic specimens (Tesarik et al, 1992). However, pentoxifylline does not modify the percentage of motile spermatozoa. In a separate study, it was concluded that an unselective use of pentoxifylline, 2-deoxyadenosine, or both compounds together may restore sperm function in some patients and perhaps improve fertilization in vitro, but in others it may produce no change or may even be detrimental to sperm function (Tournaye et al, 1994). Pentoxyfilline, in addition to stimulating sperm motility, has also been shown to induce sperm capacitation and the acrosome reaction in fresh (Ain et al, 1999) and cryopreserved sperm (Esteves et al, 1998).

In Vitro Treatment With Platelet-Activating Factor

Previous studies have shown that human spermatozoa express platelet-activating factor (PAF) receptor (Reinhardt et al, 1999) and that treatment of sperm with PAF results in a receptor-mediated improvement of sperm function (Levine et al, 2002). Furthermore, in vitro treatment of spermatozoa with PAF has been reported to significantly improve pregnancy rates in intrauterine insemination (Wild and Roudebush, 2001). A beneficial effect for PAF on sperm function in vitro is supported by the fact that sperm from a PAF receptor knockout mouse strain display a significantly reduced rate of capacitation. When used for in vitro fertilization, sperm from PAF receptor knockout mice gave a significantly lower rate of fertilization (21.5%) than did wild-type sperm (66.7%) (Wu et al, 2001).

Sertoli/Germ Cell Coculture

In vitro culture systems capable of supporting human early germ cell differentiation have been developed for treatment of azoospermic patients or patients with germ cell maturational defects. Sertoli cells, spermatogonia, and spermatocytes, isolated from testicular biopsies of azoospermic patients, have been cocultured using Vero cell-conditioned medium alone or supplemented with recombinant (r) follicle-stimulation hormone (FSH) or rFSH plus testosterone (Sousa et al, 2002). Optimal results are achieved using the hormones in combination. In vitro matured spermatids microinjected into oocytes elicit 37.5% fertilization and 28.6% blastocyst rates. Abnormal elongating and elongated spermatids resulted in 8.3% and 27.3% fertilization rates, respectively, but none of the embryos reached the blastocyst stage. Normal elongating and elongated spermatids result in 30.5% fertilization and 42.9% of blastocyst rates (Sousa et al, 2002). Fluorescence in situ hybridization analysis showed sex chromosome anomalies in all embryos except in the case of morulae from normal, late spermatids. These results suggest that meiosis and spermiogenesis can be resumed in vitro and with normal differentiated spermatids showing a low fertilization potential but regular rates of blastocyst formation. However, most of the embryos do not reach the morula stage and show major sex chromosome abnormalities (Sousa et al, 2002).

Supplementation of Culture Media With Antioxidants

Supplementation of culture media with antioxidants has been used to minimize sperm lipid peroxidation (Alvarez and Storey, 1983). More recently, oxidative DNA damage induced by 30 Gy X-irradiation was reported to be prevented by ascorbic acid (600 μM), alpha tocopherol (30 and 60 μM), and urate (400 μM) therapy. These antioxidants provided protection from subsequent DNA damage by x-ray irradiation. In contrast, acetyl cysteine or ascorbate and alpha tocopherol together induced further DNA damage. In contrast, supplementation in vitro with the antioxidants ascorbate, urate, and alpha tocopherol when used separately had beneficial effects on sperm DNA integrity (Hughes et al, 1998). These results underscore the importance of careful antioxidant application and dose selection in preventing oxidative stress.

Incubation of sperm from oligoasthenozoospermic samples at 5% O_2 has been shown to result in a significant improvement in motility parameters, the percentage of hyperactivated motility, and induced-acrosome reaction, compared with those observed after incubation in an atmosphere of 20% O_2. Exposure to 5% rather than 20% oxygen tension also induced a significant increase in the percentage of sperm penetrating zona-free hamster eggs after capacitation for 17 hours. After 24-hour incubation, a significantly higher survival rate is observed under 5%, compared with 20%, oxygen tension (Griveau et al, 1998), confirming previous reports (Alvarez and Storey, 1985; Alvarez et al, 1987). These results strongly suggest that use of low oxygen tension might improve sperm function by minimizing oxidative damage to sperm.

Use of Hyaluronic Acid

A relationship has been found between diminished cellular maturity of human spermatozoa and low-level expression of the testis-specific chaperone protein, HspA2 (formerly known as CK-MM). Because HspA2 is a component of the synaptonemal complex in rodents and assuming that this is also the case in men, it has been postulated that the frequency of chromosomal aneuploidies would be higher in immature versus mature spermatozoa (Kovanci et al, 2001). Hyaluronic acid (HA) has been shown to improve the proportion of high HspA2 and ma-

ture spermatozoa in a given sample (Sbracia et al, 1997). Therefore, the use of HA could be beneficial in assisted reproduction, eg, intrauterine insemination and in vitro fertilization (IVF). Because HA is a physiological component of the cumulus oophorus that surrounds the oocyte and of the female and male reproductive tracts, clinical use of HA should not cause ethical concerns. However, it should be emphasized that the release of mature sperm, with a normal HspA2 profile, into the seminiferous tubules does not ensure that these spermatozoa retain their fertilizing potential before they leave the testis. Mature sperm will still be susceptible to DNA damage during migration through the seminiferous tubules and the epididymis (Steele et al, 1999). This damage may not be compatible with the initiation and maintenance of a term pregnancy, and yet these sperm retain a normal HspA2 profile.

Intracytoplasmic Sperm Injection

Intracytoplasmic sperm injection (ICSI) has been shown to be an effective tool in the treatment of cases of severe oligozoospermia and oligoasthenozoospermia (which before the introduction of ICSI would require the use of donor sperm) or when sperm are unable to undergo capacitation, bind to the zona pellucida, undergo the acrosome reaction, or fuse with the oocyte's plasma membrane (Liu and Baker, 2000; Liu et al, 2003). Microinjection of spermatozoa directly into the oocyte's cytoplasm could help bypass defects in these sperm functions.

Calcium Ionophore-Induced Egg Activation

Oocyte activation is a series of events triggered by the fertilizing spermatozoon and necessary for the initial stages in embryonic development. Calcium plays a pivotal role in this process (Marangos et al, 2003). During ICSI, there is an initial considerable but short (<2 minutes) increase in $[Ca^{2+}]i$ that is detected immediately after the penetration of the microinjection needle into the ooplasm, regardless of whether a spermatozoon is simultaneously microinjected or not (Tesarik et al, 1994). Furthermore, this intracellular calcium rise, induced by the microinjection pipette, does not itself provoke oocyte activation. After a lag period of 4–12 hours, oocytes undergo a second wave of $[Ca^{2+}]i$ changes. These changes are sperm dependent and follow 1 of 2 alternative patterns, a nonoscillatory one and an oscillatory one. The nonoscillatory pattern resembles the changes described previously during parthenogenetic (micropipette-initiated) activation of mammalian oocytes. The oscillatory pattern is similar to the changes accompanying normal fertilization in different mammalian species. Therefore, the initial $[Ca^{2+}]i$ rise provoked by the ICSI procedure is not responsible for oocyte activation, and the release of a sperm factor(s) is required to initiate this process (Tesarik et al, 1994).

Oocytes can be activated in vitro following treatment with calcium ionophore A23187 and 6-dimethylaminopurine (6-DMAP) (Tesarik et al, 2000). In this procedure, oocytes are exposed for 10 minutes at 37°C to a solution of 10 μmol/L ionophore A23187 in Gamete-100 medium (prepared from a 2 mmol/L stock solution in dimethylsulphoxide), washed 3 times in fresh Gamete-100 medium, and incubated for 3 hours in IVF-50 medium supplemented with 2 mmol/L 6-DMAP (37°C, 5% CO_2 in air). Oocytes can be activated by the combined treatment with ionophore A23187 and 6-DMAP. Therefore, in those cases of failed fertilization because of sperm failure to induce oocyte activation, calcium ionophore and 6-dimethylaminopurine could be used.

Recently a novel sperm-specific phospholipase C (PLC) has been identified as the sperm factor that triggers Ca^{2+} oscillations in mouse eggs indistinguishable from those observed at fertilization. PLC removal from sperm extracts abolishes Ca^{2+} release in eggs. Moreover, the PLC content of a single sperm is sufficient to produce Ca^{2+} oscillations as well as normal embryo development to blastocyst (Saunders et al, 2002). This PLC has the potential to be used to induce egg activation in in vitro fertilization.

Selection of Nonapoptotic Sperm

Apoptotic sperm expressing phosphatidylserine (PS) on the outer leaflet of the membrane can be separated by magnetic-activated cell sorting (MACS) after binding to superparamagnetic annexin V-conjugated microbeads (ANMBs) (Paasch et al, 2003). Spermatozoa from donors show lower levels of bound annexin V and activated caspases than spermatozoa from infertile patients. MACS results in a significant decrease of spermatozoa with activated caspases in both donors and infertile patients. Separation effects of the MACS technique have been confirmed with flow cytometry using antiannexin V antibodies and electron microscopy. Therefore, ANMB-MACS removes spermatozoa with PS-bound annexin V and produces a higher-quality sperm fraction (Paasch et al, 2003).

Summary

In spite of the significant progress made in human reproduction in the last decades, there are some conditions in the male that still lack a specific treatment for infertility, eg, microdeletions of the Y chromosome (AZFa, AZFb), meiotic defects, sperm maturational arrest, aneuploidies, defective centromeres, defects in oocyte activation, etc.

Nevertheless, the formidable advances made in the field of assisted reproduction in the last decade have given us the opportunity and means to help bypass defects in

sperm function that would otherwise leave the couple as infertile. As indicated above, the introduction of ICSI has afforded the treatment of many forms of previously untreatable male factor infertility, eg, oligozoospermia, oligoasthenoteratozoospermia, azoospermia, sperm function abnormalities.

Concerning preproduction nurturing, although preventing or minimizing exposure to some of the factors described herein is known to favorably impact on testicular function and sperm quality, there are still some questions that remain to be answered. For example, can sperm production be resumed once exposure to a toxicant (eg, chemotherapy, xenobiotics) has been discontinued? Hopefully, future studies will provide answers to these questions.

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