

Testis and Temperature: An Historical, Clinical, and Research Perspective Minireview

ABRAHAM MORGENTALER, BRANDON C. STAHL, AND YIZHONG YIN

From the Division of Urology, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts.

One of the most remarkable concepts in human biology is that the male gonad requires a specialized temperature environment in order to produce sperm. Not only does spermatogenesis occur most effectively at a relatively cool temperature, but core body temperature is actually lethal for the male germ cells. Humans, as well as many other mammals, have thus developed a specialized extracorporeal pouch, the scrotum, to provide for a cool testicular environment.

Contrary to conventional wisdom, the development of a scrotum is not universal among mammals. Approximately one third of mammals maintain the testes internally, including the elephant, the rhinoceros, and the aquatic mammals, to name a few of the more commonly recognized species. A scrotum does not exist at all among birds, reptiles, amphibians, or fish. Thus, the requirement for a testicular environment cooler than core body temperature is not universal and, more accurately, represents an evolutionary adaptation for some mammals.

This adaptation comes at a significant cost. Once or twice a year at our hospital, we are presented with the clinical scenario of a ruptured testis in a young man following trauma, often requiring removal of the damaged testis. A similar situation does not exist for the female gonads since the ovaries are internal. Indeed, the testes are unique among the body's organs with regard to vulnerability: the brain is encased in the skull; the heart, lungs, liver, spleen, and kidneys are protected by the thoracic cage; and the pelvic organs are protected by the bony pelvis. In contrast, the testes hang outside the body, covered only by a thin layer of skin and muscle, exposed to a variety of physical insults as mundane as a stray branch or soccer ball.

What is the evolutionary benefit, then, of externally

located, cool testes? Although a full treatment of this issue is beyond the scope of this paper, the most compelling argument is based on studies showing that the cool scrotal environment allows for greater sperm storage within the epididymis and, thus, the ability to have a larger number of successfully fertile matings within a short period of time (Bedford, 1978). This would be of greatest benefit to the seasonal breeder, particularly in species where a dominant male mates with multiple females over one to several days. This does not seem to describe the human species but certainly may have been true for our evolutionary forebears.

Regardless of the Darwinian forces that have brought us to this state, we are faced with a remarkable biologic system with temperature as a key regulatory factor. Temperature has important implications for male infertility, for contraception, and for understanding cellular control mechanisms for male reproduction. It is the objective of this paper to provide an historical, clinical, and research perspective on the topic of testis and temperature. As with any paper of this type, the goal is to provide key concepts rather than an exhaustive literature review.

Anatomy and Physiology

Since the testes represent the male's reproductive potential, it is reasonable to expect that the organism would develop mechanisms to provide and maintain the necessary temperature environment, apart from simply creating a passive external pouch for the testes. Indeed, there are five main anatomic features that allow the testes to remain cool: 1) the dartos muscle, 2) the cremaster muscle, 3) the countercurrent heat exchange system, 4) an absent fatty skin layer, and 5) abundant sweat glands.

The dartos muscle lines the scrotum and relaxes when ambient temperature is warm. This cools the testes by causing them to lie further away from the body, which is the local heat source. Cremasteric fibers in the spermatic cord act similarly but more directly on testicular distance from the body proper. As warm blood in the testicular artery courses peripherally to the testes, some of its heat is transferred to the cooler returning blood in the surrounding veins of the pampiniform plexus. This is a countercurrent heat exchange system, causing blood in the testicular artery to become progressively cooler as it travels toward the testes. Finally, the absence of fat in scrotal

Correspondence to: Dr. Abraham Morgentaler, 330 Brookline Avenue, Boston, Massachusetts 02215. Email: amorgent@caregroup.harvard.edu

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skin allows ambient temperature to cool the testes without intervening insulation, and sweat glands contribute to heat loss by evaporative cooling.

In the 1960s, Waites demonstrated the importance of cool testicular temperature to the organism as a whole and raised the possibility of yet another cooling mechanism. He created a specialized heating device for the scrotum of the ram and discovered that scrotal exposure to 40°C for 30 minutes resulted in the animal dropping its rectal (core) temperature by a full degree centigrade. The woolen animal produced this temperature drop by panting, whereas the shorn animal achieved the same temperature reduction by vasodilation, rather than by a change in respiratory rate. Application of an equivalent amount of heat to the flank of the animal produced no change in rectal temperature (Waites 1961, 1962). The clear interpretation of this experiment is that the animal was trying to lower its temperature in an effort to maintain testicular thermostasis, at the expense of core temperature for the entire organism. This experiment underscores the importance of the cool testicular environment, and hence the reproductive potential, to the individual organism.

Historical Perspectives

In ancient Greece, it was believed that heat was related to vitality and, thus, to fertility. The man provided his to the woman during intercourse. A curious result of this logic was that a large penis was considered less desirable, since it was thought to allow the semen to cool during ejaculation and, thus, to become less fertile. More recent concepts regarding heat and fertility have been focused on the testes. Although it had been recognized for centuries that cryptorchidism among domestic animals and humans can result in sterility, the reason for this was unknown until there was recognition of two facts: scrotal temperature is lower than abdominal temperature and heat has a deleterious effect on spermatogenesis.

In the 1920s, Moore and colleagues published a series of papers that established the concept of testicular sensitivity to heat (Moore, 1924; Moore and Quick, 1924). Most important among these was the simple observation that scrotal temperature was cooler than abdominal temperature. This was determined by placing a thermometer inside the abdominal cavity of various animals and recording the temperature as the thermometer was advanced through the inguinal canal into the scrotum. Scrotal temperature varied by species but was generally 2–7°C cooler than abdominal temperature (Moore and Quick, 1924).

In 1923, Fukui performed a remarkable experiment to show that the germ cell loss seen with cryptorchidism could be explained entirely by a heat effect (Fukui, 1923). Fukui performed surgical cryptorchidism on dogs by el-

evating the testicles into the abdomen and then suturing them to the abdominal wall. He then applied an external cooling device to the abdominal wall overlying the area of the surgically transposed testes. Histology of the cooled cryptorchid testes showed preservation of normal spermatogenesis, in contrast to the spermatogenic degeneration normally seen with experimental cryptorchidism.

These experiments established the concept that cool temperature is critical for normal testicular function and that heat produces spermatogenic impairment. In 1934, Phillips and McKenzie demonstrated alterations in semen parameters in the ram after scrotal insulation raised local temperature by only 2.2°C. More recently, it has become recognized that some cases of male infertility are due to conditions where there exists testicular exposure to heat as well.

Clinical Aspects

At least three causes of male infertility are clearly related to heat: varicocele, cryptorchidism, and fever. In addition, there may be men with subfertility based on intrinsic abnormalities of scrotal temperature regulation, although this concept is less well established. Fever has not been extensively studied as a cause of infertility; however, it is well recognized among clinicians that sperm concentration, motility, and morphology may all be reduced for several months following a febrile illness.

Cryptorchidism has been recognized for centuries to be a cause of infertility in men and domestic animals. Griffiths showed in 1893 that experimental cryptorchidism caused testicular atrophy in the dog, but it remained for the experiments of Moore (1924), Moore and Quick (1924), and Fukui (1923) in the 1920s to show that the cause was heat. Of course, with naturally occurring cryptorchidism, there may be additional factors at play to explain the abnormal sperm production, such as the genetic makeup of the individual. However, there is now reasonable evidence that early correction of cryptorchidism results in improved fertility compared to late correction, indicating that there is an environmental effect of unrepaired cryptorchidism. Heat is a reasonable and adequate explanation for this observation, since the undescended testis is exposed to suprascrotal temperature and cannot take advantage of the cooling mechanisms described above.

The effect of cryptorchidism on fertility can be severe. A recent testicular biopsy in a 40-year-old man with azoospermia and a history of bilateral cryptorchidism repaired at age 20 showed a classic picture, with absence of all germ cells. Even unilateral cryptorchidism can cause significant subfertility. In one study, men who had undergone repair of unilaterally undescended testis at 4–

12 years of age had a mean sperm concentration that was only one-third as high as that of normal men (Lipshultz et al, 1976). Varicocele is the most common identifiable condition affecting fertility in man, and correction of varicocele results in improvement in the semen analysis in a majority of men.

Varicoceles are dilated veins in the scrotum that become fuller with standing position and can occasionally cause a weighty discomfort when they are quite large. However, the diagnosis is usually made on examination by a urologist when a man presents with subfertility. Varicoceles were first noted by Celsus (born in 25 AD) to cause some degree of testicular atrophy: when the disease has spread also over the testicle and its cord, the testicle sinks a little lower and becomes smaller than its fellow, inasmuch as its nutrition has become defective (Zorgniotti, 1991).

What is the relationship between varicocele and temperature? Both intratesticular and scrotal temperature have been shown to be higher in men with varicoceles, compared with men without varicocele (Goldstein and Eid, 1989). In addition, scrotal temperature rises in men with varicocele as they change position from supine to standing, whereas scrotal temperature drops in men without varicocele when they stand from a supine position. Larger varicoceles were associated with a greater change in temperature, and surgical correction of the varicoceles abolished the rise in temperature with upright position (Yamaguchi et al, 1989). For the group with a large varicocele, the mean difference in temperature prior to corrective surgery was 2.0°C.

The cause of the elevated temperature with varicocele requires an understanding of the regional anatomy. The pampiniform plexus of veins carries blood from the testis through the spermatic cord, coalescing into the testicular vein(s), which travel upwards in the retroperitoneum to finally drain into the renal vein on the left and the inferior vena cava on the right. When valves are absent or incompetent in the veins, gravity pulls blood down into the scrotum, causing the veins to become dilated. The blood coming from the retroperitoneum is at core temperature, and reflux of this relatively warm blood likely contributes to the scrotal warming seen with varicocele. This is a dynamic event, and one can watch the temperature rise with a scanning thermometer as a man with a large varicocele stands up from the supine position. During correction of the varicocele, the veins are ligated and cut, thereby interrupting the reflux of warm blood down into the scrotum.

Although most of the attention regarding heat and testicular function has been focused on the germ cells, there is now evidence that varicoceles cause a reduction of serum testosterone levels, since there is improvement following varicocele repair (Su et al, 1995). This raises the

possibility that some of the effect of heat on spermatogenesis is mediated by altered Leydig cell function, perhaps by lowering intrascrotal testosterone levels.

There is additional evidence that some men may have an intrinsic defect in scrotal thermoregulation that predisposes them to infertility. When comparison of scrotal temperatures was made in men with excellent sperm parameters and in those with oligospermia without varicocele, it was found that 80% of men in the oligospermic group had elevated temperature (Zorgniotti and Sealfon, 1988). A scrotal cooling device based on evaporative heat loss was developed to treat infertile men, with some improvement in semen parameters reported (Zorgniotti et al, 1980, 1986). In another study, 50 men with oligospermia or diminished sperm motility were treated with nightly ice packs. A twofold or greater increase in sperm density or motility was seen in 65% of the men, regardless of whether a varicocele was present (Mulcahy, 1984). Larger scale studies of men with elevated scrotal temperature would be valuable to better understand the clinical impact of heat and fertility. Nonetheless, it is clear that testicular production of sperm in normal quantity and quality is vulnerable to temperature increases as small as 1–2°C.

Heat and Male Contraception

If testicular exposure to heat causes infertility, can this relationship be adapted to provide a beneficial result, such as male contraception? Rock and Robinson (1965) showed that insulating the underwear of male volunteers resulted in a significant drop in sperm concentration and motility. Remarkably, they found that a change in scrotal temperature of only 1°C was enough to effect this change. However, the continued presence of small numbers of motile sperm argued against this treatment as a reliable form of contraception.

Mieusset et al (1985) developed a form of underwear designed to maintain the testes in the inguinal canal at night as a potential contraceptive. Sperm counts were dramatically reduced, but it seems unlikely that this form of contraception would be widely accepted by the public. However, scrotal heating may turn out to be a reasonable form of contraception if the technical challenges of convenience, comfort, reliability, and reversibility can be met. A note of caution is that rodents that undergo experimental cryptorchidism fail to demonstrate any subsequent recovery of spermatogenesis if the testes are kept in the abdomen for more than a limited period of time.

The Undershorts Controversy

One of the most commonly asked questions by couples undergoing fertility evaluation is whether the men should wear loose-fitting underwear (boxer shorts) in order to

keep the testicles cool. This concept is so widespread that many men switch to boxer shorts as soon as they wish to initiate a pregnancy. As noted above, small changes in scrotal temperature may have a significant effect on sperm production, and so the concept of cooler underwear is logical. Is there data to support this?

In a recent study, scrotal, core, and skin temperatures were measured in men presenting for infertility. No temperature differences were noted between men wearing tight or loose underwear, and the authors concluded that there was no need for men to change to boxer shorts for fertility purposes (Munkelwitz and Gilbert, 1998). However, in a prospective, randomized study volunteers wore loose or tight underwear for 6 months and then crossed over to the other type for the next 6 months (Tiemessen et al, 1996). Sperm concentrations were nearly twice as high for these men when they wore loose underwear compared with when they wore tight underwear. Motility was improved in the loose-underwear group as well.

Although no definitive answer to the undershorts controversy is yet available, patients clearly benefit from the ability to take an active role in treating their medical problems, and in this light, the recommendation to wear loose-fitting underwear can be therapeutic on several levels. However, it must be emphasized to clinicians and patients alike that a change in underwear is unlikely to be the solution to an established fertility problem.

Basic Science Research

Since the work of Griffiths (1893) over 100 years ago, it has been established that cryptorchidism, and hence heat, causes impaired spermatogenesis. But what are the actual changes that take place? Research performed in a variety of laboratory mammals consistently shows that the primary spermatocyte and early spermatids are the germ cell stages most vulnerable to heat. In practical terms, the testis is seen to shrink in size and histology shows thinning of the germinal epithelium with loss of the affected germ cell stages. Vacuolization, representing gaps in the epithelium from absent germ cells, may be seen with heating and is also a feature of testicular biopsy in men with varicocele. Neither Sertoli nor Leydig cell numbers appear to be reduced after testicular heating. The major result of testicular exposure to suprascrotal temperature is loss of germ cells.

In the early 1920s, Moore (1924) showed that 30 days of experimental cryptorchidism in the guinea pig resulted in complete loss of the germinal epithelium except for Sertoli cells and some spermatogonia. This work indicated a relative resistance to heat of spermatogonia compared to other germ cell types. Complete recovery of germinal cryptorchidism continued for 4 months. This may have been because of an irreversible effect of prolonged heat exposure, or the lack of recovery may have been

because of loss of the remaining spermatogonia. Once the stem spermatogonia have been lost, there is no further capability for spermatogenesis to recover.

One of the first cellular manifestations of heat exposure is condensation of nuclear components, termed pyknosis, in spermatocytes and early stage spermatids and formation of multinucleated giant cells (MGC) (Moore, 1924; Clegg, 1963; Davis and Firlit, 1966). Pyknosis has been shown to be a typical morphologic feature of apoptosis (Wyllie et al, 1980). Apoptosis is now recognized to be a widely practiced form of cell death in biologic systems, and it is often termed cell suicide because the dying cell brings about its own demise in an energy-dependent manner. It is to be distinguished from necrosis, which is a passive, externally mediated form of cell death. Recently, it has been shown that the loss of germ cells in response to heat occurs via apoptosis.

The use of the unilaterally cryptorchid model in the mouse has proved useful for looking at the effect of temperature on the testis. In this model, one testis is brought up into the abdomen and sutured to the abdominal wall, while the contralateral testis is similarly manipulated into the abdomen but is then replaced in the scrotum to act as an euthermic control. Serum testosterone and gonadotrophin levels have been shown to undergo minimal changes, if any, in this model (Gomes and Jain, 1976). Curiously, the weight of the cryptorchid testis in the mouse is unchanged for 6 days but then loses weight precipitously beginning on day 7, so that there is a 40% reduction in weight by day 10, and a 55% reduction by day 14. Histology reveals positive staining for apoptosis by day 7, as well as loss of primary spermatocytes and vacuolization. Testes from day 10 and 14 reveal further loss of round spermatids, with relative sparing of spermatogonia and, in some tubules, spermatozoa. Molecular techniques, however, revealed the presence of apoptosis by characteristic DNA fragmentation beginning on day 6. Scrotal testes showed no evidence of weight loss or apoptosis (Yin et al, 1997b).

The longest stage of spermatogenesis occurs with the pachytene spermatocytes, during which time chromosome crossover and DNA exchange between sister chromosomes takes place. Pachytene cells must therefore have the capability to repair large numbers of DNA strand breaks in order to ensure the integrity of the genome for subsequent reproduction. Significantly, the tumor suppressor gene p53 is highly expressed in the testis (Rogel et al, 1985; Almon et al, 1993), with the greatest level in pachytene spermatocytes (Schwartz et al, 1993). Since p53 is known to cause cell cycle arrest as well as to induce apoptosis in response to DNA damage, we postulated that p53 might play a role in heat-induced germ cell apoptosis.

Mice lacking p53 were subjected to unilateral crypt-

orchidism and demonstrated delayed testicular weight loss and apoptosis beginning on day 10, whereas wild-type animals underwent apoptosis by day 7 (Yin et al, 1998a). This 3-day delay indicates that heat-induced germ cell apoptosis occurs via a minimum of two pathways, with a p53-dependent pathway responsible for the early apoptosis and a p53-independent pathway responsible for subsequent apoptosis. Apoptosis that is p53 dependent has generally been associated with DNA damage (Lowe et al, 1993; Fisher, 1994). It is likely that the relative susceptibility of various germ cell stages to heat injury correspond with differing intracellular levels of molecular agents involved in apoptotic pathways.

p53 appears to be involved in quality control for germ cells, since p53-null mice have been shown to have lower levels of spontaneous apoptosis, greater abnormalities of sperm morphology, and reduced number of offspring when mated with wild-type females (Yin et al, 1998). Germ cells must have a mechanism to sequester p53 in order to avoid apoptosis during normal replication. It has now been shown that p53 is localized to the nuclear envelope during normal spermatogenesis and translocates to the nucleoplasm with heat-induced apoptosis (Yin et al, 1997a).

It is unclear why there is a 6–7 day delay in germ cell apoptosis following experimental cryptorchidism in wild-type mice (Yin et al, 1997b). In another experimental model in which testicular heating is effected by scrotal immersion in a hot water bath, heating for 15 minutes at 43°C induces germ cell destruction within 24 hours (Chowdhury and Steinberger, 1970). In contrast, similar treatments at lower temperatures of 36–41°C failed to produce germ cell loss. This suggests that there may be a minimal time–temperature dosage of heat required to induce germ cell apoptosis, perhaps based on a threshold level of DNA damage (Steinberger and Dixon, 1959). This time–temperature dosage may be different for various germ cell stages. Although the delay in apoptosis seen in the cryptorchid model may be due to a necessary interval before manifestation of an initial heat injury, it seems more plausible that the constant exposure to a relatively mild temperature increase eventually reaches the time–temperature threshold for apoptosis hypothesized here.

A time–temperature threshold may also play a role in a clinical scenario, in which men undergoing infertility assessment are generally discouraged from partaking in episodic heat exposure, such as hot baths or saunas. In rats exposed to water baths of 40°C three times weekly for 30 minutes for 4 weeks, there was a clear reduction in fertility, as assessed by a mating assay (Loughlin et al, 1991). Although not directly evaluated, it is likely that sperm concentration was reduced due to germ cell apoptosis. Since a single exposure of 30 minutes at 40°C does

not induce germ cell loss, this experiment supports the concept of a time–temperature dosage requirement for apoptosis. In a human experiment, subjects took sauna baths eight times over 2 weeks, raising the mean core temperature by 0.93°C after staying in the baths for a mean of 15 minutes. Mean total sperm number dropped from approximately 160 to 68 million by day 35 following initial heat exposure, with rapid recovery to baseline levels (Procope, 1965). It thus appears that intermittent heat exposure causes impaired spermatogenesis in humans as well.

Is germ cell apoptosis in the cryptorchid testis a direct result of abdominal heat stress, or is it a secondary effect of an altered intratesticular hormonal pattern? Supporting the endocrine pathway to apoptosis are the data showing that testicular testosterone concentration is reduced in the cryptorchid testis (Keel and Abney, 1981; Sharpe et al, 1986) and that testosterone withdrawal induces germ cell apoptosis (Tapanainen et al, 1993; Troiano et al, 1994). On the other hand, *in vitro* experiments have shown that heat can cause germ cell apoptosis even in the face of a hormonally controlled environment (Yin et al, 1998b). *In vivo* experiments in bilaterally cryptorchid mice show that apoptosis precedes the reduction in intratesticular testosterone (Ohta et al, 1996). Taken together, the data suggest that there is a direct effect of heat on germ cells, with an additional contribution from reduced testosterone levels.

Apart from direct induction of apoptotic triggers, the precise role of other biochemical agents in the temperature sensitivity of the testis remains to be elucidated. Enzymes such as acid phosphatase, lactate, and succinate dehydrogenases have all been shown to be affected in the testis by mild hyperthermia (Blackshaw et al, 1973). DNA polymerases alpha, beta, and gamma, as well as topoisomerase I are also reduced in activity at 37°C, compared with 31°C, in an *in vitro* model (Fujisawa, 1997). However, it is not clear whether these changes in enzymatic activity contribute to the heat-mediated loss of germ cells or merely reflect impaired cellular function. Of great interest is that there appear to be testicular versions of some proteins that function best at the cooler scrotal temperature. For example, cholesteryl ester hydrolase has a testicular isoenzyme that is temperature labile and has somatic isoenzymes that are temperature stable (Wee and Grogan, 1993). In addition, heat shock factor is activated in the testis at a lower temperature than in the liver. It has been proposed that this lower set point for heat shock factor activation in the testis may be related to a reduced thermal stability of testicular proteins (Sarge et al, 1995).

Summary

The testis is remarkable as a biologic system for its functional regulation by temperature. Not only does the testis

function optimally at a relatively cool temperature, but core body temperature is lethal to germ cells. This temperature sensitivity has implications for clinical medicine, both in terms of understanding pathologic states and for therapeutic measures. Perhaps most important, the relationship between testis and temperature presents great opportunities for further elucidation of cellular control mechanisms, particularly with regard to gametogenesis.

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