The Possibilities of Clinical Transplantation of the Testis as an Organ and Tissue

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In our century the transplantation of testes as replacement therapy and the method of rejuvenation of the organism have elicited great interest. The history of the procedure started with the transplantation of testes from adult men; then came transplantation of fetal testes (with the description of various new surgical methods), followed by experiments in the transplantation of human fetal testicular tissue, including tissue cultures, and treatment with lyophilizates from animal testes. The transplantation of the fetal testis as a tissue and as cell cultures and treatment with testicular tissue extracts may effectively stimulate the regeneration of the testis proper in hypogonadism of various etiology, including incurable hypergonadotropic forms.

Key Words: *transplantation of the testis; transplantation of testicular tissues; treatment with lyophilizates from testicular tissue*

The science of transplantology started in 1767, when Hunter transplanted testes in roosters [20]. The classic investigations of Berthold (1849) [36] and Brown-Sequard (1889, 1892) [45] in the transplantation of testes in roosters and mammals showed that some active substance, later on called hormone, travels from the transplant through the blood to other organs, providing for normal male sexual development and behavior. This initiated the appearance of the new science of endocrinology. Simultaneously Brown-Sequard laid the foundation for clinical transplantations when he administered to himself extract of testes of guinea pigs and dogs.

The new therapeutic method gained acceptance in Russia at once, and the results of the first investigations were reflected in the works of Viktorov (1891) and Predtechenskii (1894) and later Guaze (1907) [3,12,25].

While the heart, kidney, and other organs were transplanted only as substitutions, the testis was widely considered not only as an organ of reproduction and male potency, but also as a life-giving elixir of youth. The first experimental investigations of Steinach in the early 20th century [41] became the topic of the leading editorial in the *Journal of the American Medical Association*, which contained the following declaration: "Great possibilities in the application of these findings loom up but it is too early for anything more than interesting speculations" [32]. A series of later publications confirmed the vast possibilities of the new method. Lispinasse (1913), Morris (1916), Lydston (1916,1918), Stanley (1920), and many others described a significant improvement in the condition of the patient of senile and presenile age treated with tissue testicular transplants. The new method was quickly accepted in Russia, where Zavadovskii (1922), Voronov (1923-1930), Brodskii (1923), Shustov (1924), Smirnov (1925), Gorash (1926), Kirov (1928), and other clinicians [1,6,15, 69] performed the same grafts not only for the treatment of potency disorders, but also in sexoneurosis and homosexuality, as well as for the general rejuvenation of the organism. Although his-

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tological studies of removed transplants demonstrated their total necrosis, the recipients described a subjective improvement not only of sexual function but also of the memory, vision, appetite, and general wellbeing.

The isolation, determination of the structure, and synthesis of the male sex hormone testosterone in 1935 [51], as well as its successful use for replacement therapy of hypogonadism, plus ethical reasons resulted in the prohibition of xenotransplantation. In fact, untiltoday, testicular transplants carry an air of scandal about them in the USA and Western Europe; only a few cases of such an operation have been performed and then only as autografts and only when absolutely necessary (traumatic castration, anorchism of one of twins) [50,61].

In Russia the opinion that the free transplantation of testes had no prospects was shared by Nemilov, who stated in his monograph (1940) that this "rejuvenation" was useless. In contrast, Rumyantsev [27], on the basis of numerous experimental and clinical investigations performed in the 40s-50s, concluded that tissue therapy, including testicular tissue, improved the course of wound healing and other pathological processes in the organism. The same view was shared by Filatov and co-authors [31].

The rise in the efficacy of testis transplantation was attributed by many investigators to the restoration of the circulation in the transplant. The first transplantation of the testis as an organ using a vascular pedicle was performed in the USSR by Frumkin in 1947 (3 cases). In the 60s-70s, in contrast to their Western colleagues Soviet physicians continued their attempts at testis transplantation, perfecting the method of the ortho- and heterotopic transplantation on vascular pedicles. Karpukhin (1964-1970), Gnilorybov and Nekhvyadovich (1965), Kirpatovskii (1970-1986), and others transplanted testes and demonstrated that such grafts can have a replacement function [5,7,8,18,20]. The transplant manifests an androgenic activity, but after 2 weeks it is rejected due to autoimmune processes, as was confirmed by Attaran *et al.* [33] and Gorbatyuk and Petrosyan [9]. The causes of failure of the early testis transplantations became evident, namely 1) insufficient asepsis and contamination of the patients with infections from the donors; 2) the difficulties with vascular anastomoses when the testis was transplanted as an organ; 3) immunological reactions of rejection. The first two problems were solved, and the technique of testis transplantation on vascular pedicles in the reconstruction of the vas deferens allows for the preservation not only of the androgenic, but also of the spermatogenetic function, as was demonstrated during isotransplantation in experiments [54] as well as in the clinic [48,61]. But immunological reactions still remain a practically insurmountable barrier [2]. In contrast to the case with somatic tissues and organs, the immunosuppression used in transplantation of the testis causes damage to the spermatogenetic function and the chronic use of glucocorticoids, chorionic gonadotropin, and testosterone often damages the endocrine function, thus substantially limiting the clinical possibilities of the method.

Certain hopes were roused when testes of nonpubertal donors were used.

Since the experimental investigations of the 30s it has been established that allografted testes of nonpubertal animals are tolerated significantly better [4,26,29,68]. The studies of Larkin [53] and Buck [38] performed in the 60s on animals testified that the transplantation of fetal testes causes a reaction of tissue incompatibility of lesser intensity than the transplantation of other organs of the same period of gestation. For this reason they are tolerated, and grow and differentiate [9-11,14]. Petrosyan and Raitsina noted [24] that spermatogenesis in a nonpubertal allograft in rats has sometimes progressed to the production of sperm. The lesser immunogenicity of allografted fetal and neonatal testes as compared to pubertal testes has been confirmed in the last 10 years by experimental investigations of Donahoe *et al.* (1984), Statter *et al.* (1988, 1989), and Barksdale *et al.* (1991) [34,64,65]. It was shown in the last study that testes of 3-day-old donor rats transplanted under the kidney capsule better preserve their structure, grow better, and are less subject to lymphocyte infiltration due to the increased level of gonadotropins. This is in agreement with the observations of Gotsiridze [11]. According to Barksdale *et al.* [34], the preservation of allografts under hypergonadotropic stimulation was the same as in isotransplantation and the use of immunosuppression with optimal doses of cyclosporin A. The immunoprivileges of fetal testes in autotransplantation are attributed by Raitsina [10] and Gotsiridze [11] to the absence in them of mature spermatogenetic cells, which possess pronounced antigenic properties. Statter *et al.* [65] consider them to express mRNA of the major histocompatibility complex to a lesser degree and therefore to produce less of the corresponding proteins. Barksdale *et al.* [34] think that organospecific growth factors, which display high activity, may be important in preserving graft viability and should be considered as a possible adjuvant in organ transplantation.

Kirpatovskii and Gorbatyuk (1987,1990) [21] were the first to undertake allotransplantation of fetal testes on an arteriovenous pedicle to boys aged 2.5-6 years old with anorchism and congenital hypoplasia of the testes (4 cases). Their attempts were more successful than when they previously used testes from mature donors [2,20]. These authors showed the relatively normal development of secondary sexual characteristics in the young recipient during 6-10 years of observation, although acute rejection of the graft occurred in one case and all recipients required immunosuppressive therapy. Isolated attempts to transplant of fetal testes with vascular connections are being continued up to the present, although it is evident that spermatogenetic function does not recover.

Another possibility is to transplant the testis as a tissue, i. e., without preservation of the anatomically intact organ and without restoration of the vascular and nerve connections. The ability of pieces of testicular tissue to become engrafted after transplantation in the scrotum, anterior chamber of the eye, ear, and other sites was demonstrated back in the 30s-40s [4,50,69,71]. Experimental studies of recent years demonstrated that homogenate from syngenetic testes [37,43], heterotopically transplanted neonatal testes [34,64,65], as well as a culture of isolated interstitial endocrinocytes [66,67] are able to compensate to a great degree for androgenic insufficiency in castrated rats for a long time. The replacement effect was noted in transplants of various localization (subcutaneous and intraperitoneal) but the most favorable place for the preservation of the functional activity of an auto- and isograft proved to be outside of the barrier, namely, intramedullary and intratesticular (this has also been shown for the transplantation of pancreatic islet cells [55,59,70]). The long-term preservation of autograft viability without immunosuppression has become possible using fetal and neonatal testes placed under the kidney capsule (see above) and transplanting a culture of neonatal endocrinocytes under the tunica albuginea, as was recently shown by Dendeberov [13]. Unfortunately, no assessment of the physiological activity of the transplanted cells was performed in the last case.

Attempts at clinical transplantation of a culture of interstitial endocrinocytes (Leydig's cells) were performed recently in Russia for the treatment of impotence and sterility. Methods have been proposed for obtaining cell cultures of endocrinocytes of animal and human testes, including fetal and neonatal testes, as well as procedures for their preparation and transplantation [17,22]. Several hundred such operations have been performed at the Center of Male Reproduction and the Center of Andrology and Transplantation of Endocrine Organs of the Russian Peoples' Friendship University (Moscow), but their results have not yet been presented in major scientific publications. The efficacy of such an operation is not in doubt in primary hypogonadism with androgen deficiency, but in other types of sterility and impotence it may be useless, because testosterone, the main product of transplanted endocrinocytes, is usually not a limiting factor in the clinical course of these disorders.

Another possibility has to do with the administration of testicular extracts. Since the 70s it has been known [40,46] that extracts of testes of pubertal animals inhibit *in vivo* the regeneration of spermatogenous epithelium after x-irradiation, treatment with cytostatics (myelosan), and hemicastration and promote the proliferation of fibrous tissue. In contrast, extracts of nonpubertal testes stimulate the proliferation of spermatogonia and androgen production [47]. Feig *et al.* [42] assumed that the effect of extract from nonpubertal testes was due to the existence of various growth factors in it. The efficiency of the use of growth factors and stimulators of regeneration is confirmed by numerous experiments with the damage of the skin, retina, and other organs [16,52]. The data of recent years [35,49,58] testify that many growth factors (IGF-I, IGF-II, TGF β1 and 2, EGF, and others) and cytokines (IL-1 α and β, and others) play a huge role in the development and paracrine regulation of testicular function. It is evident that extracts of tissues of the prenatal period, when the processes of organogenesis occur and pronounced proliferative activity is present, contain an abundance of different growth factors. The administration of a complex of them must produce a stimulating effect on the regeneration of pathologically altered organs. This idea underlies cell therapy, the most renowned advocate of which is Schmid [60]. The author and his co-workers succeeded in stimulating the regeneration of many tissues using lyophilizates of various tissues, including nervous tissue. There have been attempts to use this therapy in male sterility and impotence [39,44,56, 60]. It has been shown that the administration of lyophilizates of fetal tissues may stimulate impaired spermatogenesis and androgen production. However, such data are scant and contradictory and contain no accurate qualitative assessment of the diagnostic and clinical findings. Schmid uses animal tissues in all cases, because it is considered that many regulators of cell activity possess organo- but not species specificity [46,57]. At the same time, there

is no doubt that the clinical use of human fetal tissues should cause a more pronounced specific effect, with less expressed immune reactions, than animal tissues. Likewise, the transplantation of living secreting cells may be more effective than the administration of lyophilizates. This was confirmed in andrology by the discoveries of Turchin and coworkers (1989), who proposed a method of treatment of male hypogonadism [28]. The method is based on the transplantation of a 2-5-day cell culture of testes from newborn suckling-pigs. Such cell transplants administered subcutaneously in 20 patients caused an increase of testosterone production by 40% and of gonadotropins by 60%, and improved the spermogram parameters by 89%. Since elevated levels of gonadotropins and testosterone do not always affect the spermogram and actual fertility [62,63], the effect is probably related somewhat to growth paracrine factors as well, which are actively produced by testicular cells in the early postnatal period.

The idea of using the high activity of juvenile cells for the stimulation of pathologically weakened functions of the organism is reflected in the German patent "The organization of a bank for autologous cell therapy" [30], where it is proposed to cryoconserve extracorporally different cells of the body. After a certain period, chosen by the donor himself, those cells possessing potential activity at the moment of sampling, are reactivated via thawing, cultured, and administered back to the donor organism as a cell suspension.

From the analysis of the literature presented, it may be assumed that the allotransplantation of fetal and neonatal testis as a tissue, cell cultures, and tissue extracts may stimulate the regeneration of the testis proper in hypogonadism of different genesis, including the usually incurable hypergonadotropic forms. Cryoconservation, preparation of cell suspensions, and culturing when necessary permit the administration to the patient organism of a complex of growth factors, other natural species- and organospecific biopolymers, and viable secreting fetal cells, which may accomplish a long-term replacement function due to their low immunogenicity. Simultaneously they may on the paracrine level have an effect on the damaged intercellular relationships and stimulate pathologically altered cells of the recipient (our experience shows that more than 10% of cells preserve their viability after cryoconservation and thawing).

There are many unsolved problems in the transplantation of fetal testis as a tissue, and the study of it is actually only just beginning. What disorders would be effectively treated by such transplantation? What is more effective - a native preparation or a cell culture, functioning cells or their products? What age of donor is optimal? What is the role of tissue- and species specificity? What factors mediate the integral and local effects? The study of these and many other aspects connected with the clinical use of transplantation of human fetal tissue for the treatment of male sterility and other disorders of male reproductive function is underway at the International Institute of Biological Medicine using the latest methods.

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