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PCA3 a promising urine biomarker for prostate cancer diagnosis

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After more than 15 years from the introduction of total PSA in the clinical practice, it appears mandatory identifying new biomarkers able to distinguish with greater specificity cancer from non-cancer patients and reduce potential risks related to unnecessary prostate biopsies.

Among the new biomarkers under development for the diagnosis of prostate cancer, the Prostate Cancer 3 (PCA3) gene is considered as the most promising. Discovered in 1999, the PCA3 gene is segment of non-coding messenger ribonucleic acid (mRNA) from chromosome 9q21-22 that is overexpressed by more than 95% of all prostate cancers tested. At tissue level, this biomarker is expressed in prostate cancer tissue 66-100 times more than in normal prostate tissue and 140 times more than in benign prostatic hyperplasia [1,2]. Moreover, this marker is not expressed in non-prostate cancers [3].

Studies of PCA3 expression in prostate tissue are limited because of the inadequacy of a tissue-based diagnostic strategy in routine practice and most of the clinical studies evaluate PCA3 in urine.

Prostate cells are believed to be present in urine, especially in the first voided sample after digital rectal examination (DRE). Urine samples should be collected after a standard protocol considering firm pressure (sufficient to depress the prostate surface by approximately 1 cm) from the base to the apex and from the lateral to the median line for each lobe. Three strokes are to be performed for each lobe. The PCA3 test requires collection of the first 20 to 30 mL of voided urine after a digital rectal massage. Interestingly, without prostate massage the test provides valid results in only approximately 80% of cases. This percentage increases to more than 98% using the digital transrectal prostate massage.

Because no protein product has been detected from PCA3 RNA, PCA3 assays were developed using RNA detection methods: 1. specific isolation of the RNA of interest using coated magnetic beads; 2. amplification, and 3. detection and quantification of the isolated RNA.

The studies measuring the expression of PCA3 in urine also evaluated the number of PSA mRNA copies. The measurement of PSA mRNA

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further allowed for the standardization of the number of PCA3 RNA copies by calculating the ratio of PCA3 to PSA ("PCA3 score"). Currently in the clinical studies a PCA 3 score (PCA3-mRNA/PSA-mRNA) has been estimated. The more accepted "decisional" cut-off value to indicate a prostate biopsies is a PCA3 score > 35 [4].

In 2003, Hessels et al for the first time demonstrated the possibility to translate the prostate cancer specificity of PCA3 at the tissue level into a specific test for disease diagnosis. Dosing the PCA3 on urine samples of 108 patients who underwent prostate biopsies for suspected prostate cancer, the authors reported sensitivity of 67%, specificity of 83%, positive predictive value (PPV) of 53% and a negative predictive value (NPV) of 90% [5].

Data coming from a recent systematic review and meta-analysis showed that test sensitivity ranged from 46.9 to 82.3%; specificity from 56.3 to 89%; PPV from 59.4 to 97.4% and NPV from 87.7 to 98%, respectively [4]. The most relevant clinical scenarios in which the PCA3 score could be tested and potentially used are: 1. in the first biopsy setting, where the serum total PSA are in the gray zone between 2.5-10 ng/mL; 2. in the re-biopsy setting, in patients with persistent suspicious of prostate cancer and negative previous prostate biopsy.

In patients with previous negative prostate biopsy and persistent elevated PSA levels, the PCA3 showed sensitivity ranging from 47 to 58%; specificity from 71 to 72%; a PPV from 39 to 43%; and a NPV from 78 to 83% [6-8]. Moreover, Haese et al. demonstrated that PCA3 score of 35 was superior to free/total PSA ratio (cut-off 25%) for predicting repeat prostate biopsy outcome [8]. This results supported the potential use of PCA3 in this setting of patients.

Few data are available regarding men with an elevated total PSA level (2.5-10 ng/ml) who did not receive any previous prostate biopsy. Regardless of the clinical setting (first biopsy *vs*. repeated biopsy), sensitivity, specificity, PPV and NPV of the test in the subgroup of patient with PSA in gray zone were 53-84%; 71-80%; 67-78% and 80-83%, respectively [9].

Concerning the potential ability of the PCA3 score to distinguish between indolent and clinically significant prostate cancer, data emerging from the literature are conflicting. Some studies demonstrating a correlation with Gleason score, tumour volume and pathological extension of the primary tumour. Conversely, two other studies failed showing any statistically significant correlation between PCA3 score and pathological features [9].

PCA3 gene is a very promising new biomarker for prostate cancer. Its score seems not to be influenced by potential confounding factors such as prostate volume, benign prostatic hyperplasia or prostatitis. Moreover, the test is not influenced by the age of the patients and provides a supplemental diagnostic information in comparison with total PSA. Although the available studies supported the use of this biomarker mainly in patients with persistent suspicious of prostate cancer who had undergone previous negative prostate biopsies, the results of a large registration trial, currently being conducted by the manufacturer (Gen-Probe, Inc, San Diego, CA), must be waited before to support a widespread diffusion of the test in the clinical practice.

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Genitourinary tract inflammation in couples and the role of ROS

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Key words

Reactive oxygen species • Infertility • Urogenital tract infections

Summary

Article discusses the role of reactive oxygen species (ROS) in urogenital tract infections. In particular regarding following issues: a. importance of the topic; b. methods of determination of ROS in semen; c. steps involved in gamete biofunctional damage; d. clinical models of infertility in couples due to post-inflammatory radicalic pathology; e. some forms of idiopathic infertility.

Considerations and importance of the topic

The reactive oxygen species (ROS) are products of a normal energetic oxidative cell metabolism, predominantly mitochondrial, as a result of reactions of enzymatic reduction of oxygen [1]. From a physiological point of view, a low level of ROS plays an important regulating role (mediating conception) [2] in various reproductive stages, both in the female (follicologenesis, oocyte maturation, oocyte quality, endometrial cycle, luteolysis, implanting, embryogenesis and pregnancy) and in the male (spermatic capacitation, acrosomal reaction, binding to the zona pellucida, sperm-oocyte fusion) [3]. Higher amounts of ROS provoke disorders in the redox potential which in turn cause oxidative stress (OS), that is to say, unbalanced overproduction of ROS and/or low antioxidising scavenger activity. The same cell sites that generate ROS at low concentrations in physiological conditions concur with other cells (leukocytes, macrophages/spermiophages), triggered by bacterial infection and/or by the dynamics of a chronic inflammatory reaction, to instigate an overproduction of ROS. Genitourinary tract inflammation in couples indicates a post-bacterial condition that is prevalently sub-acute or chronic, or post-inflammatory involving one or more tracts of the reproductive apparatus concerning the male (epididymis, prostate, seminal vesicles) or the female (tubes, endometrium, uterine cervix, vagina) gonad. Several aspects of this topic have caught the attention of researchers: the superimposable age-group involved, the symptomatological aspects and functional sequelae (infertility). In the male, in the sex glands of the urogenital tract, a chronic inflammatory reaction determines an altered redox balance due to the excess of pro-oxidative mediators

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(bacterial infection, cytokines, ROS) and/or reducing anti-oxidising capacity of the enzymatic and biological (non-enzymatic) systems [3-5]. This outcome, known as oxidative stress (OS), manifests in biofunctional disorders of the spermatozoa, resulting finally in possible male factor infertility. In the presence of ROS overproduction, the peroxidative damage spreads over a greater area of membrane, thus constituting *in vivo* the factor responsible for limiting survival of spermatozoa in the female reproductive apparatus [6] as well as interfering *in vitro* with several of the spermatic bio-functional stages, (acrosomal reaction, oocyte penetration assessed with sperm penetration assay) [7]. The phases of transport up to the fertilization site in the female genital tract host a complex of stages of bio-functional damage to the spermatozoa mediated by the ROS which are not always possible to assess and which, given the importance of this area of research, undoubtedly warrant further investigation. In the female there are some so-called "biological windows" [8] represented by follicular fluid, tubaric fluid and peritoneal fluid, that offer in some clinical models the possibility to analyse the redox metabolic balance (ROS; TAC) at such levels: the presence of oxidative stress may transmit and/or amplify (in the case of a concomitant male radicalic pathology) functional damage to the spermatozoa, impairing induction of pregnancy both in the case of natural conception and of medically assisted procreation [9].

Methods of determination of ROS in semen

The "total" measurement of ROS (intra and extracellular) in semen is analysed with the technique of chemiluminescence (expressed in photons per minute, cpm) using as a probe "Luminol" that is sensitive to a wide range of ROS with a neutral pH value, or "Lucigenina" that is more suitable for determining the extracellular quota of ROS [10]; for the highest assessment of ROS overproduction of leukocytary or iuxta-sperm origin, fMLP or PMA stressors are added [10]. The method of flow cytometry represents another option for the measuring intracellular quota of ROS, deriving mainly from the alteration of the mitochondrial respiratory chain [11]. Spectrophotometry is used for the measurement of malondialdehyde (MDA), that is the final outcome of the processes of peroxodisation of the polyunsaturated fatty acids in the sperm membrane lipids. Also the total antioxidising capacity (TAC) of the seminal plasma can be assessed with chemiluminescence by means of kinetic reading, expressed in Trolox equivalent molar values [13].

Steps involved in gamete biofunctional damage

In a clinical model of overproduction such as in the case of urogenitary inflammation, the ROS condition all those events which precede and which follow the fertilization: 1) chemotactic migration phase of the spermatozoon towards the oocyte; 2) induction of the acrosomal reaction; 3) binding with the oocyte zona pellucida; 4) interaction and fusion with the oocyte oolemma; 5) oocyte activation. Acquisition of the human spermatozoon's competence to fertilize involves a series of events, including chemotaxis which plays a key role. Capacitation is a transient state (1-4 hrs in vitro) towards which single spermatozoons move at different times, thus giving rise to a continuous renewal of capacitated cells [14] with a view to guaranteeing availability of spermatozoa that are fit for fertilization for an extended period of time [15]. The spermatozoa acquire their chemotactic responsiveness as part of the process of capacitation [16]. Chemotaxis consists of directional modulating of the movement of the spermatozoa in response to a gradient of chemoattractant molecules. In the female reproductive apparatus, once the spermatozoa arrive in the tubes they are detained in the isthmus that carries out the function of a reservoir. At the moment of ovulation, some capacitated spermatozoons detach themselves from the tubaric isthmic region in response to the chemoattractant molecules released by the oocyte itself and/or by the cells of the cumulus oophorus. Thus, this guarantees the constant availability of subpopulations of capacitated spermatozoa, capable of responding to chemotactic stimuli which guide the spermatozoa towards the oocyte [17]. The role of chemotaxis in humans is that of recruiting capacitated spermatozoa, accounting for 2-14% of the total spermatic population [18]. A series of molecules present in the follicular fluid (FF) of various species of mammals, including humans, are considered potential chemotactic agents for the spermatozoa, traditional but controversial such as progesterone [19], more recent such as RANTES, a β-chemokine soluble found in various fluids of the genital tract and the in FF [20].

Clinical models of infertility in couples due to post-inflammatory radicalic pathology

MAGI – this acronym groups together a set of infections of the male accessory glands (Male Accessory Gland Infections) and features among the recognized diagnostic categories as a cause of male factor infertility [21]. The micro-organisms responsible for MAGI and for genital inflammation in the couple can originate in the urinary tract or can be agents of sexually transmitted diseases [22].

Some bacteria (Gram-negative, enteropathogens, *Ureaplasma urealyticum*, *Chlamydia*), alone or together with their membrane products (LPS from Gram-negative germs or from *Chlamydia*) and toxic metabolytes such as H_2O_2 and NH_3 produced by *U. urealyticum*) [23] contribute to overproduction of ROS [24 25]. Few studies have concentrated their research on the correlation between types of germs, oxidative stress and sperm quality. In vitro, after coincubation of spermatozoa from normozoospermic subjects with one of 5 bacterial strains (*Escherichia coli, Staphylococcus haemolyticus, Streptococcus oralis, Bacteroides ureolyticus, U. urealyticum),* the analysis of intermediate products of ROS (ROI) and of MDA revealed a significant increase in MDA after exposure to *B. ureolyticus, S. haemolyticus or E. coli* [24]. Nevertheless, the prevailing opinion is that oxidative stress is secondary not so much to the *noxa infettiva* as to the chronic inflammatory reaction of the accessory sex glands [3-5 25]. The secretory dysfunction of the glands, triggered by bacterial and/or inflammatory infection, represents the most important cause of oxidative stress and is expressed through an aspecific chronic inflammatory reaction (leukocytospermia, increase of proinflammatory cytokines in seminal plasma: interleukin IL-1, IL-6, IL-8, tumor necrosis factor TNFα; overproduction of ROS) and/or a specific reaction (overproduction of autoAb antinemasperm) [3-5]. These bioactive substances may persist even after an apparent post-antibiotic suppression of bacterial growth, thus prolonging the presence of OS insomuch as the initial antioxidising and scavenging capacity, sustained by the nonenzymatic micronutrients of the epididymal plasma, deteriorates progressively in the course of the chronic inflammatory process. The biochemical damage induced by the radicalic pathology determines a cascade of negative biofunctional effects of the spermatozoon, such as damage to the DNA and/or apoptosis [3]. Moreover, a chronic secretory dysfunction negatively influences the morphofunctionality of the glands concerned in the inflammatory process. In reference to this question, infertile subjects manifesting MAGI with bacteriospermia > 105 CFU/ml or with infection from *Chlamydia* or *U. urealyticum* presented an elevated number of ultrasound alterations (cases of more extended MAGI) on the transscrotal didymus-epididymis ultrasound (TRUS) scans, prostato-vesiculo-epididymitis (PVE) and orchi-epididymitis [4 25]. Patients also presented an elevated chronic inflammatory reaction in terms of leukocytospermia and ROS overproduction, and a parallel alteration of the principal spermatic parameters directly correlated to the extension of the MAGI (prostatitis < prostato-vesiculitis < prostato-vesiculo-epididymitis) [4].

Hydrosalpinx

This regards occlusion of the fimbriated end of the tube near to the ovary, caused by liquid retention, specifically hydrosalpinx fluid (HSF) in the site, followed by swelling and distension which provoke the loss of its anatomical shape and its functionality. This presents a post-inflammatory or post-bacterial condition of OS insomuch as it demonstrates: a) elevated production of ROS and MDA; low antioxidising activity of the total oxidizing capacity [26]; b) the tubaric fluid originating from the hydrosalpinx has been shown in a murine experimental model to have embryotoxic properties. The development of blastocysts was inversely correlated to the percentages of HSF added to the murine two-cell embryo (in contrast with controls of murine two cell-embryos without HSF, grown in culture up to the manifestation of blastocysts), and correlated positively with the production of ROS. Other factors concur to the state of OS and the embryotoxic properties of the hydrosalpinx, germs, such as endotoxins, cytokines and loss of micronutrients which provide antioxidising activity [27]. In contrast, an influx of hydrosalpingeal fluid from a previous hydrosalpinx without overproduction of ROS would seem to indicate a different type of damage from that of the endosalpinx, with positive prognostic prospects for pregnancy after in vitro fertilization [9], insomuch as there appears to be metabolic recovery in the cells of the tubaric epithelium.

Some forms of idiopathic infertility

Some forms of idiopathic infertility that is not explained by the concurrence of male and/or female factors may be interpreted as conditions of oxidative stress, when for instance in the peritoneal fluid of females in such a condition a) lower concentrations of antioxidising capacity (TAC) are present in comparison with control groups represented by fertile women or females with infertility due to tubaric causes [28] and b) elevated levels of ROS [2]. The hypothesis that emerges from this is that the peritoneal fluid spreading in the tubes causes damage to the spermatozoa that are notoriously sensitive to oxidative stress [29].

Recent updates on the topic and future prospects

Hopes of finding markers capable of predicting lack of fertilization other than the conventional seminal parameters are still considered rather utopian in the

light of the complexity of this process. Sperm membranes of mammals are rich in unsaturated fatty acids and are sensitive to the damage induced by the oxygen mediated by lipid peroxidisation [30]. In physiological conditions, seminal plasma possesses antioxidising mechanisms that are capable of neutralizing the ROS and which offer protection from damage that may occur to the spermatozoa. Recently, a lipid molecule physiologically present in the seminal plasma has been discovered, the endocannabinoid palmitylethanolamide (PEA), which derives from palmitic acid. It has been suggested that substances such as PEA may regulate numerous physiological processes in the reproductive systems, modulating the capacitation, affording protection against infection and maintaining cell vitality [31]. Moreover, it has been demonstrated that PEA and other acylethanolamides are oxidizing molecules involved in the protection of spermatozoa from environmental damage [32]. As well as continuing to investigate leukocytary and spermatic aspects of this topic, future research seems to be directed towards the mitochondrial compartment of the spermatozoa inasmuch as the membranes of these organelles are rich in phospholipids with a high degree of unsaturation [30].

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Conservative surgery for the treatment of penile carcinoma

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Summary

The rationale for organ preserving surgery in the treatment of squamous cell carcinoma (SCCA) of the penis is based on three key concepts. 1. About 80% of penile carcinomas occur distally, involving the glans and/or prepuce and thus are potentially amenable to organ preserving surgery. 2. Patients with penile carcinomas stages Tis, Ta, T1; grades 1 and 2 are at low risk for local progression and/or distant metastatic spread. 3. The traditional 2 cm excision margin has been challenged as unnecessary for patients undergoing partial penectomy for SCCA. Conservative techniques with surgical margins of less than 10 mm appear to offer excellent oncological control. Circumcision, laser ablation, Mohs' micrographic surgery and partial or total glansectomy associated with various forms of reconstruction have been reported as surgical organ-preserving procedures for the treatment of SCCA. The aim of the present article is to give an overview on the results and indications of penile sparing surgical techniques in the treatment of penile cancer.

For carcinoma in situ (also referred to as erythroplasia of Queyrat or Bowen's disease) laser ablation has been employed successfully.

In case of lesions limited to the foreskin, wide local excision with circumcision is sufficient primary curative therapy, although care must be taken to ensure adequate clearance margins are achieved. If the tumor has involved the glans, the choice of therapy is dictated by tumor size, extent of infiltration, and degree of tumor destruction of normal tissue. All these factors can indicate if organ preservation is a reasonable alternative to amputative procedures. Total and partial glansectomy, with or without grafting procedure, produce good cosmetic and functional results with minimal morbidity without sacrificing cancer control.

Key words

Penile cancer • Circumcision • Laser ablation • Mohs micrographic surgery • Glansectomy • split-thickness skin graft

Introduction

Penile cancer is an uncommon malignancy with a reported annual incidence of 0,9-1 in 100,000 of the male adult population in Europe and in the USA. Ninety-five percent of penile tumors are squamous cell carcinomas (SCCA) and 78% of all tumors are diagnosed on the glans and/or prepuce [1 2].

Historically, options for management of invasive penile cancer have included amputative surgery or radiotherapy.

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Amputative surgery, based on partial or total penile amputation and closure of the penile stump using the skin of the shaft to the urethral mucosa, provides excellent local control rates greater than 90% of the primary tumor and, therefore, remains the oncological "gold standard" for all stages SCCA [3 4]. Nevertheless, this approach invariably leads to considerable cosmetic deformity, psychological traumas and functional loss with resulting sexual and urinary dysfunction with more than 50% of patients developing mental disorders [5-7].

Radiotherapy is an organ-preserving technique but has been used with limited success. Indeed, SCCA is relatively radioresistant and it requires a relatively long treatment regimen of 3 to 6 weeks and a large radiation dose of 6.000 cGy [8]. The local recurrence rate has been reported in over 45% of cases [9]. Almost all patients have acute radiation reactions with necrosis which can lead to amputation of the penis, urethral stenosis, urethral fistula, chronic penile pain and penile oedema that lead to a not negligible drop out rate from the treatment. Moreover, around 40% of patients have chronic radiation reactions that can make harder the early diagnosis of a potential local recurrence [8-12].

The limits of the radiotherapy treatment and the justifiable reluctance of many patients to undergo a mutilating penectomy has promoted the use of penile sparing surgical techniques to maintain penis function and appearance [13 14]. The aim of the present article is to give an overview on the results and indications of penile sparing surgical techniques in the treatment of penile cancer.

Rationale for organ preserving surgery

The rationale for organ preserving surgery in the treatment of SCCA of the penis is based on three key concepts:

1. about 80% of penile carcinomas occur distally, involving the glans and/or prepuce and thus are potentially amenable to organ preserving surgery [15];
2. patients with penile carcinomas stages Tis, Ta, T1; grades 1 and 2 are at low risk for local progression and/or distant metastatic spread [16];
3. the traditional 2 cm excision margin has been challenged as unnecessary for patients undergoing partial penectomy for SCCA. Conservative techniques with surgical margins of less than 10 mm appear to offer excellent oncological control [17 18].

Therefore, until more rigorous scientific evidence is available, organ-preserving strategies should be reserved to well-selected patients with low-grade, low-stage disease [15], as in these cases the achievement of good cosmetic and functional results should not compromise long-term oncological outcomes. Circumcision [15], laser ablation [19], Mohs' micrographic surgery (MMS) [20] and partial (PG) or total glansectomy (TG) associated with various forms of reconstruction have been reported as surgical organ-preserving procedures for the treatment of SCCA [1 2 21].

The goals of penile-preserving treatments are to maintain penile/glans sensation and to maximize penile shaft residual length when possible.

Circumcision

The majority of men with penile carcinoma are uncircumcised. Small low-stage (Tis, Ta, T1) and low-grade (grades I and II) tumors limited to the distal prepuce can be managed by circumcision alone, with a 2 cm margin of clearance and this procedure can be considered the standard treatment in these clinical settings [13]. If the tumor is closer to the coronal sulcus, the circumcision margin must be extended proximally to the penile shaft to ensure adequate oncological resection, as recurrence rates may be as high as 50% [22]. A careful selection of the patients and the use of intraoperative frozen sections are imperative to reduce local recurrence rates.

Laser ablation

Laser energy for penile lesions was first introduced by Hofstetter and Frank in 1980 [23]. The carbon dioxide laser and neodymium YAG laser are the most commonly used in current practice [19 24]. The carbon dioxide laser vaporizes tissue with minimal penetration of energy into the deeper layers (only 0.1 mm) as the energy is completely absorbed at impact by the water component of the cell and therefore it is unsuitable for most tumors with a recurrence rates of up to 50% [24]. The neodymium YAG laser penetrates tissue and causes coagulation to a depth of at least 3 to 4 mm [24].

Overall recurrence rates with neodymium YAG laser ablation are stage-dependent. Using this laser, Malloy et al. on 16 treated patients, 5 Tis, 9 T1 and 2 T2 carcinomas, had no recurrences in all patients with TIS, while 33% of the T1 patients and all T2 patients showed local recurrence, at a follow up that ranged between 12 and 36 months [25]. Frinberger et al. in 29 patients, at a mean follow-up of 47 months, had a 5.8% incidence of local recur-

rence in Tis patients and a 10% recurrence rate in T1 patients [19]. Von Eschnbach et al. reported good outcomes for T1 tumors with excellent cosmetic and functional results and high satisfaction rates. Recurrences were noted in 6.9% of patients, which is comparable to recurrence rates after partial amputation (0-8%) [26].

In a recent retrospective study, Meijer et al. reported the results obtained in 44 patients (21 T1, 17 T2 and 6 Tis) after a mean follow-up ranging between 3 months and 16 years. Local disease recurrence in the treated area occurred in 48% of the patients, and in 20% of them, the first recurrence was elsewhere on the glans penis. In 10 cases, nodal metastases were found and 8 of these cases were stage T2. The authors concluded that laser therapy is best for stage Tis and T1 tumors exclusively. Only selected patients with T2 tumors should be treated in combination with early groin lymph node resection, as, with respect to the risk of nodal metastases, the T2 stage represents the stronger prognostic predictor [27].

Overall, these results taken together, demonstrate that laser surgery, who has significant cosmetic and functional advantages over traditional amputation, is feasible. Neverthless, patient selection is extremely important as only those tumors invading less than 4 mm into tissues are suitable for neodymium YAG laser treatment. Therefore, this treatment is best reserved for superficial and small lesions. It is most suitable for Tis lesions and should be done always in conjunction with frozen-section biopsies. It may also be performed in T1 patients, but a close surveillance is mandatory during follow up for early detection of tumor recurrence. A proper surgical procedure should be suggested for T2 patients, unless in very selected cases and in these patients every local procedure should be associated with groin lymph node dissection.

Mohs micrographic surgery

The microscopically controlled surgery represents a reasonable compromise to control local disease while providing organ preservation in patients with small superficially invasive lesions. The tumor is excised, in multiple sessions, in layers and each layer is examined microscopically by systematic frozen sections. Excision is continued until the excised tissue is negative at the histological examination. Reported complications include glans disfigurement and meatal stenosis [28]. Mohs reported 35 cases in 50 years of experience, with a local recurrence rate, at 5 years follow up, of 14% for T1 lesions, 18% for T2 and 100% for T3 lesions [20].

Brown et al presented the results in 20 patients: 11 with SCCA, 7 with SCCA in situ, 1 verrucous carcinoma and 1 leiomyosarcoma. At a mean follow up of 3 years, local recurrence was noted in 6% and a lymph node recurrence in 24% of SCCA patients. One patient died of metastatic disease and another died of unrelated causes [28].

Bissada and coworkers reported the results on 30 patients: 17 underwent inguinal lymphadenectomy which revealed lymph node disease in 12. At a follow up ranging between 12 and 360 months, 3 local recurrences and 1 cancer related death were observed [15].

Finally, Shindel and coworkers treated 33 patients who underwent a total of 41 Mohs procedures (26 Tis, 4 T1, 7 T2 and 4 T3). Follow up data was available on 25 patients at a mean follow up of 58 months. Eight patients had local recurrence (32%), which was managed by repeated MMS in 7 and by penectomy in 1. There were 2 cases of tumor progression, including 1 from T1 to T3 disease (meatal involvement) and 1 from T1 to inguinal lymph node involvement. Two patients died, of whom 1 had no evidence of penile cancer and 1 had metastatic disease [29].

Overall, taken these results together, MMS can allow for the local complete excision of the tumor with preservation of local penile anatomy and function. However, local failure can occur and therefore this technique should be reserved only for patients with small, distal, superficially invasive tumors [29].

Partial glansectomy

There are three kinds of PG reported in the literature:

1. PG with primary glans closure;
2. PG with graft reconstruction of the glans;
3. PG without grafting.

PG is indicated in localized tumors of the corona or central glans with no surrounding carcinoma in situ or obvious erectile tissue involvement on MRI.

Primary glans closure is suitable for small and isolated lesions. For larger lesions, when primary closure is not technically feasible or might not be oncologically safe, various techniques have been suggested to cover the area. McDougal performed partial glans excision in five patients. All underwent wide excision of the disease, preserving the urethral meatus [30]. In 2 patients a full-thickness graft was used to resurface the hemiglans defect and in 1 a split-thickness graft was applied to the corona

and glans area [30]. Ubrig described a simple technique in which an outer preputial skin flap was used to cover the glans defect [31]. Pietrzak and coworkers suggested the use of a full-thickness flap of penile skin or extragenital (lateral aspect of the thigh) split thickness skin graft [2]. The subtotal glans excision without grafting, reported by Ralph [1], consists in carrying out two circumferential incisions: the first is a skin incision at the level of the coronal sulcus, and the second around the meatus, both with a macroscopic clearance of ≥ 5 mm and confirmed by frozen-section analysis. The tumor and healthy glans, between the incisions, are then excised leaving the urethra intact. The residual glans along with the urethral meatus is then sutured down to the distal corpora and the penile skin closely approximated to it with absorbable sutures. This technique preserves the meatus and gives a better cosmetic and functional outcome. Of the 5 patients treated by Ralph, the postoperative stage and grade were T1G2 and T1G3 in two patients and T2G2 in one patient. At a mean follow-up of 12 months, no patients have had a clinical recurrence, or had voiding difficulties. These men, sexually active before surgery remain so, with cosmetic and functional results acceptable to the patients [1].

Overall taken together, these results showed that, if technically feasible, PG is an effective treatment for penile tumors allowing for a radical removal especially in case of T1 lesions.

Total glansectomy

The surgical technique of TG, first described by Austoni in 1996 [32], consists of a circular incision in the distal shaft skin down to Buck fascia. At this level, a plane is developed to separate the glans from corporal tips. The urethra and the neurovascular bundle are isolated in their distal extremities. The glans is dissected from the corpora cavernosa and the urethra is distally sectioned. The use of multiple frozen sections of the surgical margins is strongly suggested. After removing the glans, the urethra is ventrally opened and the neomeatus fixed to the tip of the corpora cavernosa. The neurovascular bundle is fixed to the albuginea proximally to the neoglans with absorbable sutures.

For distal tumors invading the tunica albuginea and/or corpora cavernosa, a more extensive resection is required and glansectomy must be associated with distal corporectomy and reconstructíon of corporal heads. TG without or with distal corporectomy are usually combined with reconstruction of a new glans using a split thickness skin graft (STSG). The STSG is harvested from the inner thigh close to the groin to improve cosmesis with the use of a dermatotome, and then tailored and transplanted to cover the tip of the corpora cavernosa. The graft is repeatedly and multiply incised and fully quilted with the use of multiple Polyglactin interrupted stitches over the top of the corpora cavernosa. Humid compressive dressing can be applied and suture-fixed on covering the penis [21].

Devis et al. treated 3 patients with verrucous carcinoma, angiosarcoma and a melanoma respectively. No patients had a local recurrence and all had normal urinary and sexual function [14]. Hatzichristou and coworkers treated 7 patients with verrucous carcinoma. At a mean follow-up of 65 months only one had local recurrence and all patients had normal urinary and sexual function [33].

Pietrzak and colleagues performed 10 partial glansectomy, 21 glansectomy with reconstruction and 8 glansectomy with distal corporectomy and reconstruction. At a mean follow up of 12 months of the patients who had PG, one had a tumor recurrence on the residual glans. None of those who had the glans removed had tumor recurrence [2]. In our series of 15 patients, treated with TG, between March 2003 and January 2008, only one patient had inguinal lymph node metastases 18 months after surgery, while none had local relapse and our crude disease specific survival rate was 93.3% after a mean follow up of 36 months. All patients were able to maintain their sexual activity starting from 3 months after surgery with a range between 2 and 6 months [21]. Orgasm and ejaculation were preserved in all patients though reduced glans sensitivity was reported by all patients as a predictable consequence of glans amputation [21].

Although the majority of series reports the use of a STSG, some experiences are based on glans replacement with buccal mucosa free grafts [34] and recently Gulino et al. has reported on a new technique of glans reconstruction using the distal urethra, with good functional and aesthetic results [35].

In conclusion, results reported in the literature showed that TG associated with resurfacing is an oncologically sound procedure for pT1 penile tumors and if associated either with distal corporectomy or distal corpus spongiosum excision it can be used also for the treatment of pT2 lesions. Indeed, conventional therapy for pT2 tumors would be a choice of penile amputation of varying degree but these patients can now be offered glansectomy as an alternative.

Discussion

Carcinoma of the penis is a rare tumor with considerable geographical variation [36 37]. The majority are SCCA (95%) and overall 78% of all tumors originate on the glans, coronal sulcus or foreskin. PG or TG, that provides excellent local control rates greater than 90% of the primary tumor, remains the oncological "gold standard" for all stages [1 2]. Therefore, the patient with penile cancer is not only challenged by the fear of suffering from a deadly disease, but is also confronted with the threat of losing a part of or the complete penis. The first questions of patients upon hearing the diagnosis usually deal with erectile function and body image, and only secondarily with the disease itself. The justifiable reluctance of patients to undergo a mutilating penectomy has prompted the search for new penis-sparing surgical techniques to maintain penis function and appearance; thus moving from the maximal tissue and organ destruction to the minimal therapeutic tissue removal [12-14]. This represents an oncological and surgical multidisciplinary evolution that has previously been witnessed for chemotherapeutic therapies where medicine moved from the maximal tolerated dosage to the minimal therapeutic dose. Moreover, the "sparing" concept, which has also been proposed for other urological diseases such as kidney cancer, is not peculiar of urologists but represents a surgical multidisciplinary issue being prioritized also in many other malignancies such as melanoma and breast cancer. Circumcision, laser ablation and micrographic surgery aim to remove the diseased tissue, but recurrence of the disease may occur in unrecognized premalignant foci arising within the unstable epithelium following a partial procedure. Circumcision is indicated as the standard treatment in small tumors of the foreskin. However, proper patient selection and attention to intraoperative frozen section margin status are imperative for a successful outcome, because circumcision alone, especially with tumors proximal to the coronal sulcus, may be associated with a recurrence rate of 50% [22]. The laser treatment offers excellent cosmetic results, with low local recurrence rates for Tis tumors [24 38]. It may also be performed in T1 patients but a close surveillance is mandatory during follow up for early detection of tumor recurrence [24 38]. Conversely, in stages greater than T1, local recurrence and poor control of the disease are reported [39]. Micrographic surgery, despite good cosmetic results, has failed to gain wide acceptance due to its elevated local recurrence rate also for T1 tumors, and moreover it is very time consuming.

PG and TG [1 2 30 40 41] are based on a surgical doctrine in which resection margins of 2-3 cm are not necessary to achieve local oncological control [8 13]. Conventional amputative surgery based on this dogma is associated with unsatisfactory cosmetic results, significant reduction in penile length and thus difficult or impossible vaginal penetration with severe consequences on patients psychology and it has been reported that more that 50% of these patients develop mental disorders [42]. On the contrary, the use of glansectomy in selected patients with carcinoma localized on the glans penis has been proposed to maintain a satisfactory penile length and to improve patients quality of life [14 40 42]. Moreover, it is an oncologically safe procedure. Many reports have shown similar 5-year disease specific survival rates for amputative procedures, reducing the width of the resection margins [14 17 42]. Moreover, if tumor recurrence occurs it is usually systemic at the inguinal lymph nodes. Cancer specific survival rates are similar to those reported using the conventional surgical treatment [21 43 44].

In patients who undergo glansectomy with reconstruction of a new glans, using a STSG, functional results are satisfactory [21]. Thus, the good aesthetic appearance of the penis and functional results that consist in good erectile function, preserved orgasmic sensation and stand up micturition, have a positive psychological impact on the patient and improve the patient's quality of life [45]. The most important disadvantages in the reconstruction of a new glans, using a STSG, are the reduced glans sensitivity, the spraying of urine when voiding and the complications of a more technically complex procedure, including graft failure and infection [21].

To overcome the disadvantages of the reduced sensitivity, a technique of glans reconstruction, using the distal urethra, have been proposed [35]. To avoid the drawbacks of TG and of the grafting procedure, Ralph suggested the use of a subtotal glans excision with the preservation of the urethral meatus and without the need of skin grafting. In such cases, a catheter remains in situ for 24 hours and the patient is discharged the next day. No men had voiding difficulties in his study and the patient sexually active before surgery remains so, with cosmetic and functional results acceptable to the patients [1].

Conclusion

Approximately 20% of patients with penile cancer are under 40 years of age and radical procedures, especially partial or total penectomy, may be psychologically devastating. The ideal surgical procedure

should eliminate the disease and preserve sexual and urinary function. This is still not always possible because of the extent of disease. Circumcision, laser therapy, microscopically controlled surgery, total and partial glansectomy have all been used in an attempt to provide organs sparing alternatives. For carcinoma in situ (also referred to as erythroplasia of Queyrat or Bowen's diseas) laser ablation has been employed successfully.

In case of lesions limited to the foreskin, wide local excision with circumcision is sufficient primary curative therapy, although care must be taken to ensure adequate clearance margins are achieved. If the tumor has involved the glans, the choice of therapy is dictated by tumor size, extent of infiltration, and degree of tumor destruction of normal tissue. All these factors can indicate if organ preservation is a reasonable alternative to amputative procedures. Total and partial glansectomy, with or without grafting procedure, produce good cosmetic and functional results with minimal morbidity without sacrificing cancer control.

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Androgens exert direct neuroprotective effects on the brain: a review of pre-clinical evidences

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Summary

Objective. To review available data describing pathways directly modulated by androgens that underlie brain neuroprotection.

Material and methods. A literature search was performed in July 2010 using the commercially available Medline online engine search to retrieve studies (from 1991 to 2010) on the mechanisms mediating the role of androgens on brain neuroprotection. A combination of the following medical subject headings was used: androgens, brain, neuroprotection, androgen receptor (AR).

Results. Direct androgen-dependent signaling pathways are active in both neuronal and glial compartments within the brain. Within physiological ranges, androgens promote β-amyloid and protein tau homeostasis, enhance antioxidant mechanisms, promote neuron viability and survival, control reactive gliosis, neuronal excitability and modulate water homeostasis.

Conclusions. Androgens directly exert neuroprotective effects in the brain thus counteracting neurodegenerative diseases and improving recovery after injury. These evidences provide rationales for androgen replacement therapy in hypogonadal subjects for the prevention, the therapy or the control of progression of neurodegenerative and neurotoxic diseases.

Key words
Androgens • Brain • Neuroprotection

Introduction

The brain is a well recognized target tissue for androgens [1]. The metabolism of androgens in the brain is quite complex. Peripherally synthesized testosterone (T), mainly the bioavailable form, crosses the blood-brain barrier thanks to its lipid-permeable nature. However, brain T levels don't completely parallel circulating levels due to multiple factors: sex-hormone binding globulin, hormone transport across the blood brain barrier, and the presence of steroid converting enzymes in the brain. Moreover, the brain can directly synthesize T and other neurosteroids [2]. T acts on neurons and glial cells within multiple sex-hormone sensitive brain areas through both direct androgen pathways (by itself or after 5α-reductase conversion to the nonaromatizable dihydrotestosterone (DHT)) and indirect pathways (following aromatization to estradiol or by acting on the hypothalamic-pituitary-gonadal axis) [3]. T and DHT can elicit their effects via either classic genomic, or rapid non-genomic mechanisms [4]. In the classic

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genomic pathway, T and DHT bind to the cytoplasmatic androgen receptor (AR) thus inducing receptor dimerization and translocation to the nucleus, where it interacts with androgen response elements on target genes and regulates their transcription. Rapid androgen pathways may be mediated or not by AR and induce a variety of cell signaling cascades, including calcium influx and activation of protein kinase A (PKA), protein kinase C (PKC), and mitogen-activated protein kinases (MAPK) [5]. Moreover, DHT may be further converted to 3β-Diol (5α-androstan-3β, 17β-diol) by the combined actions of the enzymes 3α-hydroxysteroid dehydrogenase (3α-HSD), 3β-hydroxysteroid dehydrogenase (3β-HSD) or 17β-hydroxysteroid dehydrogenase (17β-HSD) [1]. 3β-Diol exerts its effects mainly binding to the estrogen receptor (ER). However, it can exert direct effects thanks to the interaction with the $GABA_A$ receptor too [1]. In the brain, ARs are expressed by both neurons and glial cells and are mainly found in the thalamus, hypothalamus, hippocampus, amygdala and cerebral cortex where they often co-localize with ERs [6]. Significantly lower AR levels have been found in the cerebellum. Beyond expressing intracytoplasmatic ARs, glial cells have been also found to express membrane-associated ARs [7].

Androgens significantly contribute to several functional and morphological aspects of brain across the lifespan. Early T exposure during prenatal and early post-natal life is involved in brain neuroplastic changes that underlie the development and the differentiation of a masculine pattern of neural and glial organization [8].

During adolescence, T is involved in the activation of preformed brain structures leading to sexually dimorphic physical behavioral and cognitive effects.

In adulthood, T exerts neuromodulatory functions that contribute to maintain brain structural and functional homeostasis [1].

With ageing, bioavailable plasma T levels significantly decrease in men [9]. Using neuropathologically normal human postmortem tissues, Rosario et al. found a robust decrease in brain T levels with advancing age with minimal values in men over 80 years of age [10]. Clinical hallmarks of brain aging include cognitive impairments such as loss of working memory, which is dependent on the prefrontal cortex and declarative (long term) memory, which is dependent on the hippocampus and other medial temporal lobe regions [11]. Moreover, ageing is associated with the development of neurodegenerative diseases, mainly Alzheimer's disease (AD). From a pathological point of view, brain ageing is associated with multiple degenerative processes including white matter atrophy, particularly in the frontal lobes, synaptic loss mainly in the prefrontal cortex and in medial temporal lobe structures and accumulation of beta amyloid protein (βA), the key factor involved in the pathogeneseis of AD [12 13].

Does a causative nexus exist between age-associated decrease of brain T levels and the reported morpho-functional impairments? Literature suggests that androgens significantly modulate specific aspects of cognition, and that androgen depletion, either through normal aging or pharmacological action, can result in specific cognitive impairments, increased incidence of neurodegenerative diseases and worse prognosis after brain injury. Moreover, animal models show that androgen deprivation causes pathological changes that parallel those age-related discussed above, and that occur in the same brain regions. Low systemic levels of free T have been associated with impaired cognitive performances in elderly men [9]. Men with a relatively higher free T index performed better on visual and verbal memory tasks and exhibited better long-term memory while those with low free T showed decreased visual memory, visuomotor scanning, verbal memory, and visuospatial processing [9 14]. In hypogonadal men, T replacement therapy has been proved to improve some cognitive abilities, mainly verbal fluency. Free T concentrations were found to be lower in men enrolled into the Baltimore Longitudinal Study of Aging who developed AD, and this difference occurred before diagnosis [15]. In men recently diagnosed with AD, T replacement resulted in improved performances on both the mini-mental status exam and the clock drawing test [16]. Rosario et al. observed an approximately 50% decrease in brain T levels in men with AD aged 60-80 years in comparison to age-matched men lacking any evidence of AD or other neuropathology, an effect that was statistically significant by analysis of covariance with age as the covariable [10]. Importantly, brain levels of T were significantly depleted in men with mild neuropathological changes to the same degree as men with severe AD neuropathology, indicating that T loss likely precedes development of AD [10].

Further evidences derive from patients treated with androgen deprivation therapy. Anti-androgen therapies used for the treatment of prostate cancer have been associated with cognitive impairments [17]. Conversely, the discontinuation of anti-androgen therapy was reported to restore cognitive performances in particular verbal memory [18]. βA levels are higher in both rodent brain and human plasma after T depriva-

tion and decrease when T or DHT levels are normalized [19 20].

Low T levels are associated with poor outcomes after acute ischemic events in men and are inversely associated with stroke severity, infarct size, and 6-month mortality [21].

Why decreased brain T levels predispose to degenerative brain alterations, cognitive impairments and worse prognosis after brain injury? Recent studies demonstrate that androgens, beyond regulating sexual behavior, exert several neuroprotective functions in the brain. Neuroprotection may be defined as an effect that may result in salvage, recovery or regeneration of the nervous system, its cells, structure and function [22]. The present review focuses on cellular and molecular mechanisms directly influenced by androgens and involved in brain neuroprotection.

Material and methods

A literature search was performed in July 2010 using the commercially available Medline online search engine. A combination of the following search terms was applied to retrieve relevant articles: androgens, brain, neuroprotection, AR. Review articles and basic studies (from 1991 to 2010) describing androgen-dependent molecular and cellular mechanisms involved in brain neuroprotection were included.

Results

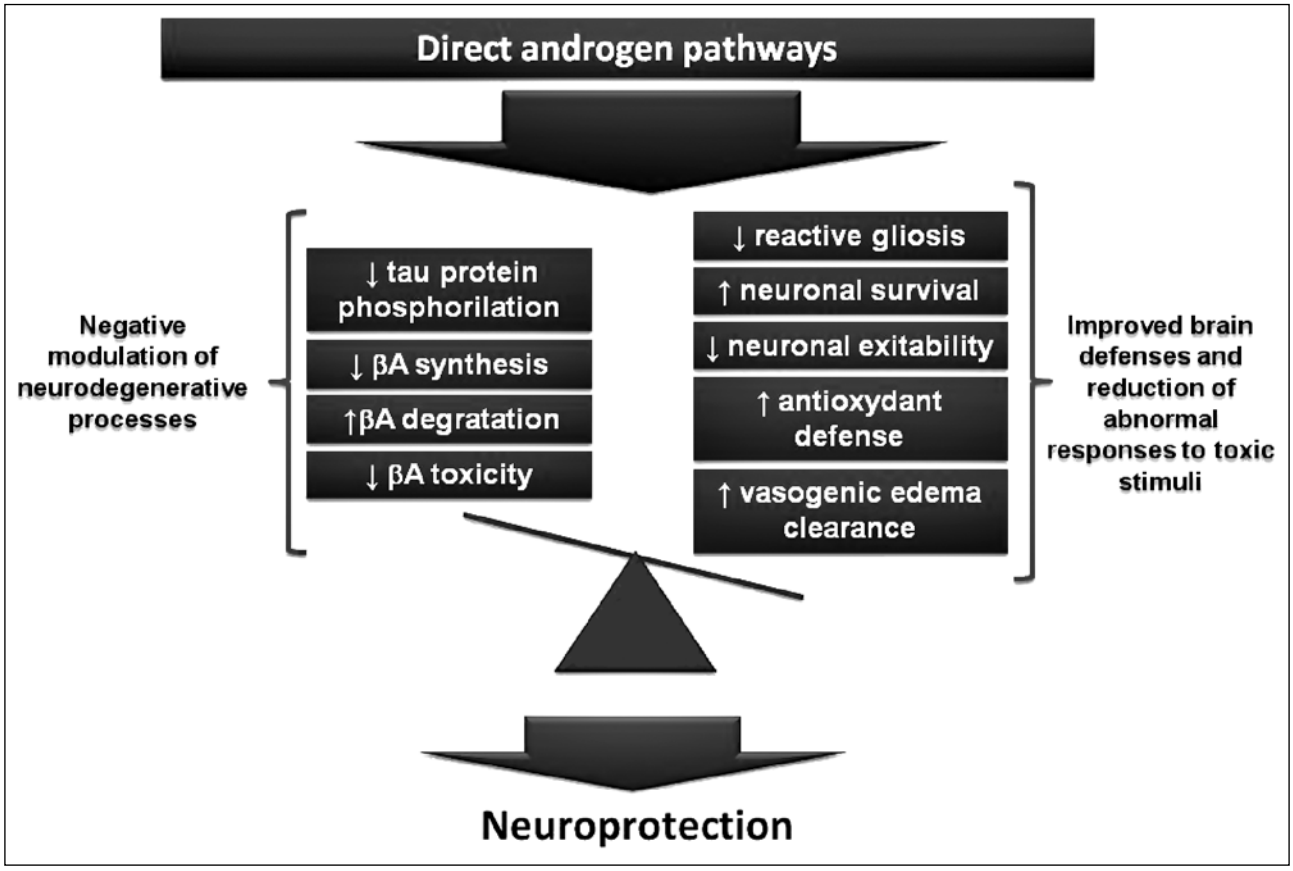
Neuroprotective pathways directly regulated by androgens have been characterized in different experimental models of brain neurodegeneration and injury (Fig. 1).

Direct androgen pathways counteracting neurodegeneration

Regulation of βA homeostasis

βA is a proteolytic byproduct of the metabolism of the amyloid precursor protein (APP), a widely expressed transmembrane protein with numerous functions ranging from axonal transport to gene transcription [23]. Brain levels of βA depend upon the balance between the opposing processes of βA generation and clearance. APP is metabolized by two competing pathways: the amyloidogenic and the non-amyloidogenic ones. During trafficking of APP along the non amyloidogenic pathway, proteolytic cleavage of APP by α-secretase re-

Figure 1. General pathways through which androgens directly promote brain neuroprotection.



sults in secretion of a non-amyloidogenic form of soluble amyloid precursor protein (sAPPα). The amyloidogenic pathway involves cleavage of APP by β-secretase at the amino-terminus of βA and release of sAPPβ. The remaining carboxyl-terminal fragment of APP is cleaved in the transmembrane domain by γ-secretase, generating βA peptides [24]. Once generated, brain levels of βA are regulated by βA clearance pathways, which include the actions of the endopeptidase neprilysin (NEP) a rate-limiting βA-degrading peptidase that largely mediates steady state brain levels of βA. Brain aging and early stage AD are associated with decreased NEP levels. Androgen response elements have been identified on NEP gene affecting NEP expression [5]. Alterations in either the production or clearance of βA that sway βA homeostasis towards increased neural levels promote intracellular and extracellular βA accumulation that abnormal assembly into oligomeric species exhibiting an altered structural conformation and capable of forming senile plaques, which are toxic for neurons, in specific brain regions. Several evidences derived from cell cultures, rodent models, and human brain demonstrate that androgens may function as endogenous regulators of βA homeostasis [25]. Androgens regulate βA methabolism through two general pathways: direct actions through AR-dependent signals, and indirect actions through estrogen pathways (after T aromatization to estradiol) or gonadotropin pathways (via T modulation of the hypothalamic-pituitary-gonadal axis) [26]. Ramsden et al. demonstrated that DHT but not 17β-estradiol administration in adult gonadectomized Sprague Dawley male rats reduced brain levels of soluble βA [19]. Mechanisms by which androgens may directly affect βA levels include both regulation of βA generation and promotion of endogenous clearance pathways. McAllister C. and colleagues crossed the aromatase gene knock-out (ArKO) mice with the APP23 transgenic mice, a mouse model of AD, thus generating the APP23/Ar (±) mice in order to study the estrogen-independent effect of T on AD [27]. Authors found, for the first time, that T administration reduced mRNA level, protein expression and activity of β-secretase in the male APP23/Ar(±) mice. Moreover, a significant increase of NEP activity was evident [27]. Furthermore, APP23/Ar(±) mice exhibited a significant reduction in brain plaque formation and improved cognitive function when compared with age-matched male APP23 controls. Further evidence for a direct androgen action on NEP expression derives from a previous study by Yao et al. demonstrating that DHT induces a time-dependent increase in NEP expression and a decrease of βA in cultured hippocampal neurons through an AR-dependent mechanism [5]. Zhang et al. demonstrated that physiological concentrations of androgens protect human primary neurons against intracellular βA peptide toxicity through AR by increasing the levels of heat shock protein 70 (Hsp70). The molecular mechanism underlying Hsp70 neuroprotection is not clear although multiple hypotheses have been formulated: p53 sequestration in the cytosol and prevention of its translocation to the nucleus and activation of apoptosis, direct interaction with the intracellular βA peptide, enhancement of steroid-mediated transcriptional activation of prosurvival genes [28].

Regulation of protein tau phosphorylation status

Protein tau is a cytosolic microtubule-associated protein that contributes to regulate the dynamism and stability of neuronal cytoskeleton by promoting microtubule assembly and stabilization. Tau metabolism is altered in several neurodegenerative diseases, as for example AD and other tauopathies. Possible mechanisms for tau alterations include abnormal phosphorylation that is responsible for the formation of aberrant tau aggregates leading to neurofibrillary degeneration [29]. Papasozomenos and colleagues demonstrated that T but not 17β-estradiol prevents tau hyperphosphorylation in ovariectomized Sprague-Dawley rats [30]. In addition, evidences suggest that T may also prevent calpain-mediated tau cleavage and the generation of the toxic 17-kDa tau fragment [31].

Direct androgen pathways involved in neuron resistance against injury

Regulation of neuron viability and survival

Ramsdem et al. investigated, in an in- vivo adult male rat model, the ability of androgens to modulate neuronal loss induced by kainate, an excitotoxin that preferentially targets the hippocampus. Authors found that gonadectomy significantly increased neuron loss induced by kainate and that DHT replacement significantly reduced the severity of neuronal loss, a finding suggesting an androgen neuroprotection exterted via an estrogen-independent pathway [32].

Hammond et al. demonstrated that physiological T concentrations were neuroprotective on human primary brain neuron cultures by protecting them against serum deprivation mediated apoptosis. This effect was mediated by AR as it was not prevented by aromatase inhibitor but was eliminated by the anti-androgen flutamide [33].

Li et al. demonstrated, in a rat model, that the injection of T immediately after hypoxia-ischemia brain

damage induced by the ligation of the left carotid common artery decreased neuronal apoptosis in the cortex and ippocampus [34].

Three main signal transduction cascades have been identified in neurons and/or glial cells that are directly modulated by androgens and that regulate cell survival: the MAPK/extracellular signal-regulated kinase (ERK) pathway (MAPK/ERK), the cAMP response element-binding (CREB) pathway and the phosphatidylinositol-3 kinase(PI3K)/Akt pathway.

MAPK/ERK cascade consists of a series of sequentially activated kinases with numerous downstream targets relevant to regulation of cell viability. Nguyen et al. found that T and DHT rapidly and transiently induced activation of MAPK/ERK in cultured hippocampal neurons as evidenced by phosphorylation of ERK-1 and ERK-2 [35]. Authors also observed that androgen mediated neuroprotection in this culture system required activation of a MAPK/ERK signaling pathway, as pharmacological inhibition of MAPK/ERK blocked both androgen-induced ERK phosphorylation and androgen-mediated neuroprotection. These effects were mediated by the rapid activation of the intracellular AR. Gatson et al. found that androgens activated MAPK signaling in C6 glial cell lines too, presumably through intracellular AR and that DHT was able to protect cultured astrocytes from cell death [36]. MAPK/ERK signaling has many downstream targets potentially relevant to apoptosis.

One downstream effector of MAPK/ERK signaling relevant to androgen neuroprotection is MAPK-activated protein kinase, also known as p90 ribosomal S6 kinase (Rsk). MAPK activates Rsk proteins by phosphorylation with subsequent Rsk-mediated phosphorylation and consequent inactivation of Bad, a pro-apoptotic member of the Bcl-2 family [1]. Other relevant effectors of Rsk that can promote cell survival include c-Fos, ER, NFκB/IκBα, Elk-1, glycogen synthase kinase and CREB. CREB is a transcription factor. In addition to being a downstream effector of MAPK/ERK signaling, CREB activity is regulated by several other signaling pathways. In neurons, CREB activation is known to regulate a variety of neurotrophic and neuroprotective effects. Pike et al. evaluated whether androgens activate CREB in primary hippocampal neuron cultures and found that androgens rapidly increased CREB phosphorylation and that this effect was dependent upon intracellular AR activation [1]. Authors also found that pharmacological inhibition of the upstream CREB signaling pathways MAPK/ERK, PI3K/Akt, PKA, or CaMKIV did not block the androgen-induced CREB phosphorylation. However, both pharmacological inhibition and depletion of PKC blocked CREB phosphorylation, suggesting a novel AR-dependent, PKC-dependent CREB signaling pathway in neurons [1]. Gatson and colleagues found that androgens activate PI3K signaling in C6 glial cell lines, presumably through intracellular AR. Upon evaluating the effects of membrane-impermeable DHT-BSA conjugates they found an opposite effect, namely that DHT-BSA suppressed MAPK and PI3K signaling and increased cell death. Authors hypothesized that in glial cells androgens can exert opposing effects on cell signaling pathways and cell viability depending upon whether they act preferentially on membrane or intracellular receptors [36].

Enhancement of endogenous antioxidant mechanisms

Oxidative stress is involved in the pathogenesis of different neurological and neurodegenerative disorders such as AD, Parkinson's Disease and Amyotrophic Lateral Sclerosis. Ahlbom et al. demonstrated, in a murine model, that cerebellar granule cells treated with T were protected from oxidative stress and cell death induced by hydrogen peroxide via an AR-mediated mechanism leading to an increase of the activity of the antioxidant enzyme catalase [37].

Control of reactive gliosis

Reactive gliosis is a complex phenomenon that includes a mixture of positive and negative responses critical for neuronal survival and regeneration. Reactive astroglia maintains the integrity of the blood-brain barrier and the survival of perilesional tissues, but may prevent axonal regeneration by forming both a mechanical and chemical barrier. Increased expression of Glial Fibrillary Acidic Protein (GFAP), an astrocyte-specific intermediate filament protein, reflects reactive astrocyte hypertrophy and is often used as an index of neurodegeneration [38]. Reducing the amount of astrocyte reactivity during aging has been hypothesized to indirectly represent an increase in neuronal well being [38]. Reactive microglia exerts important positive functions by remodelling the damaged tissues, but releases pro-inflammatory cytokines and may exacerbate neuronal damage [39]. Barreto et al. evaluated the effects of early and late therapy with T or its metabolites, oestradiol and DHT, on reactive astroglia and reactive microglia after a stab wound brain injury in orchidectomized rats. Authors demonstrated that both early and delayed administration of T reduced reactive astroglia and reactive microglia and these effects may be at least in part mediated by oestradiol, while DHT may mediate part of the early effects of T on reactive mi-

croglia [40]. Moreover, T can reverse the age-related increase in GFAP in the male rat cerebellum model [38]. Pan et al. evaluated the role of T during recovery from neurological deficits in a rat focal ischemia model by demonstrating less GFAP expression and reactive astrocyte hypertrophy around the infarct area in T-treated rats compared with controls [41].

Control of brain water homeostasis

Aquaporin-4 (AQP4) is the most abundant water channel in the brain, where it is expressed in pericapillary astrocyte foot processes, glial limiting membranes and ependyma. AQP4-mediated transcellular water movement is crucial for fluid clearance in vasogenic brain edema following brain injury. Gu F. and colleagues demonstrated that T, but not 17β-estradiol can up-regulate AQP4 mRNA and protein expression in cultured rat astrocytes thus ameliorating their osmotic fragility from hypoosmotic stress [42].

Inhibition of seizure activity

A further mechanism of androgen protection involves attenuation of seizure severity and subsequent neuronal injury. In seizure-related lesion paradigms, Frye and colleagues found that acute androgen treatment reduced hippocampal damage [43]. Their data suggests a protective mechanism that involves inhibition of seizure activity by the DHT metabolite 3β-diol [44]. 3β-diol appears to attenuate seizures by modulating $GABA_A$ receptor activity to increase chloride conductance and thereby suppressing excitatory signaling.

Conclusions

One of the less known actions of androgens is neuroprotection. Results from literature analysis demonstrate that androgens, within physiological ranges, can favorably regulate multiple molecular pathways in the brain involved in neurodegeneration and in the response to neurotoxic stimuli through direct mechanisms. In particular, T can decrease Aβ accumulation and protein tau phosporylation and can modulate vulnerability of neurons to toxic insults (i.e., ischemia, excitotoxicity). T-mediated neuroprotection is an intriguing field of interest from both pathophysiological and clinical point of views. The age-related depletion of T likely diminishes the ability of the brain to adequately regulate Aβ and tau protein homeostasis, resulting in increased risk for neurodegenerative diseases.

On the other hand, T replacement in aged peoples with hypogonadism may represent a valid strategy to prevent, treat, or control progression of neurodegenerative diseases and to improve neuron recovery after brain injury. Evidences described in the present review are in accordance with clinical studies demonstrating improved cognitive performances after T administration in old hypogonadal peoples and in patients with recent diagnosis of AD. However, limits from current pathophysiological knowledge should be taken in account when considering the neuroprotective therapeutic value of systemic T administration in humans. First of all, brain T levels don't reflect exactly serum levels due to the intense metabolism the hormone undergoes (estrogen conversion by aromatase, in-loco synthesis). Moreover, most current evidences derive from pre-clinical, animal studies and may not completely reflect human brain physiology. Modern molecular neuroimaging techniques may represent a valid tool to further investigate the level of T and of its metabolites and the androgen/estrogen receptor status within specific brain regions and in different clinical settings.

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Robot-assisted vasectomy reversal

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Introduction

In the early part of the twentieth century, the vasectomy gained momentum with the application for eugenic, punitive and therapeutic purposes. Today, with the exception of its use as prophylaxis procedure after transurethral resection of prostate or adenomectomy for benign prostatic hyperplasia (BPH) to prevent urogenital infections, vasectomy is widely used as a contraceptive tool and it is calculated that about 50 million men have relied on vasectomy for family planning. Above all in United States vasectomy is employed as contraceptive method by nearly 11% of married couples [1]. With the upcoming success of vasectomy, a new surgical procedure, the vasectomy reversal was born. The birth of the reversal procedure goes back to the work of Edward Martin, Chief Surgeon at the University of Pennsylvania during the early years of the past century, although technically he performed vasoepididymostomies in men who had obstruction secondary to epididymitis and not vasectomy. In 1902, Martin reported the first documented vasoepididymostomy in his study of 192 sterile couples and examination of sperm morphology. In 1909, he reported his first series of 15 azoospermic men who had obstructive lesions, 11 who had epididymal and 4 who had vasal obstruction. In the group of 11 he performed a vasoepididymostomy with patency and pregnancy rates of 64 and 27%, respectively. The significance of his contributions to the field of male fertility inspired Jecquier to entitle Martin the "founding father of modern clinical andrology" in her profile piece [2].

In the early years of vasectomy reversal, most people assumed that this procedure was hardly worth considering, due to the technical challenges and low success rates. Because urologists approached this relatively untested reversal procedure with trepidation due to the political, religious and personal implications of vasectomy surgery, men undergoing vasectomy reversal often did so under a cloak of secrecy [3].

Today, the rising popularity of vasectomy as a means of sterilisation combined with the continuing upward trend of divorce and remarriage, especially in industrialised countries, results in an increasing number of vasectomy reversals. Divorce and remarriage is the most common reason men seek vasectomy reversals, but many men undergoing reconstruction have the same partner and simply desire more children [2].

Key words
Vasovasostomy • Vasoepididymostomy

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It is estimated that about 6% of men who have undergone vasectomy will subsequently request a vasectomy reversal procedure [4].

For patients requiring vasectomy reversal to have their own biologically related children the potential alternative is sperm extraction for subsequent IVF or ICSI. During the counselling process the chances of success in terms of patency of vas and/or pregnancy based on the personal experience of the surgeon, patient's health history, age, results of physical examination and reproductive potential of his partner must be discussed in order to plan the more appropriate intervention to reach pregnancy.

Although vasectomy reversal is technically feasible in most men, its indications and ultimate success depend both on male and female fertility factors [5]. The age and fertility of the female partner should be considered carefully in discussions regarding prognosis for achieving a successful pregnancy after vasectomy reversal. A maternal age of > 40 years compared to 20-29 and to 30-39 years is an independent predictor of lower pregnancy rates following vasectomy reversal but still compared favourably with published pregnancy rates following ICSI in women aged 40 years or older [6]. Physical examination may reveal that a large segment of the vas deferens was removed and the presence of testicular or epididymal abnormalities. Epididymal fullness suggests obstruction at that level but may not predict accurately which will require vasoepididymostomy [7]. A predictive model based on age and time since vasectomy may be helpful for identifying men more likely to require vasoepididymostomy [8].

Vasectomy reversal outcomes can vary since there are many factors that alter the chance of success. Some of these factors become known preoperatively, whereas others can be ascertained only at the time of surgery. Preoperatively, the urologist must identify and understand the predictive value of these factors in order to properly advise the patient and his partner. The study by the Vasovasostomy Study Group showed that the decline in success of reversal surgery is a gradual downward trend over the years after vasectomy [5]. In contrast, a more recent study from Boorjian and colleagues demonstrated no change in patency rates even 15 years after vasectomy [9]. Most studies show a decrease in some way, when comparing patency rates after 5, 10, 15 and 20 years of obstructive interval. It is important to note that vasectomy reversal still yields higher success rates, when compared to IVF-ICSI rates [10]. Nearly all patients undergoing vasectomy reversal have a history of having previously fathered children of impregnated a partner. Although a history of fertility does not ensure normal spermatogenesis, the absence of previous fertility should raise concern; these patients should undergo the standard endocrinological infertility evaluation. Inguinal surgery, usually a pediatric hernia repair or a repair with mesh, may result in a second point of vasal obstruction. The true prevalence of vasal obstruction is not known. The studies that were carried out about the result of redo vasectomy reversal indicate that failure of prior reconstruction is not a contraindication to reconstructive procedures. The lower pregnancy rates after reversal surgery should be discussed with the patient.

The Vasovasostomy Study Group was the first to describe the difference in outcome of vasectomy reversal in men with the same female partner or those with new partners [5]. With the same partner, results are better. No scientific explanation has been found, proven fecundity as a couple, shorter obstructive interval and stronger emotional dedication have been proposed as possible reasons for higher success rates [11].

The importance of maternal age on pregnancy rates following vasectomy reversal has not been well defined, largely due to a paucity of data on this issue. As already mentioned, a retrospective analysis by Hinz et al. in 2008 clearly showed that a maternal age of > 40 years compared to 20-29 and 30-39 is an independent predictor of lower pregnancy rates following vasectomy reversal but still compared favourably with published pregnancy rates following ICSI in women aged 40 years and older. Careful counselling of older female partners is essential [6]. Some effort has been made to evaluate the importance of antisperm antibodies (ASA) testing before vasectomy reversal. We must conclude that the majority of men will form ASA after vasectomy. The majority of men, however, will conceive after a technically successful vasectomy reversal. These two findings suggest that determination of ASA status before reversal surgery is not recommended as it will not affect the management of the individual patient [12 13]. Surgeon skills are, more than in other techniques, of great importance and usually outcomes improve with experience and refinement of technique.

Vasovasostomy and vasoepididymostomy are challenging procedures requiring a specific and continuous training with microsurgical techniques. The shift from macroscopic to microscopic techniques during the 1980s improved success rates steadily, from patencies of 80% and pregnancies of approximately 20 to 30% using macroscopic techniques to patencies of 90% and pregnancies of 50 to 60% using

microscopic techniques [14]. Other technical changes included the use of microscopic two-layer, one-layer, microdot, stents, and oblique technique. The scar formation from inadvertently obliterating the lumen of vas with a poorly placed suture and failing to close the anastomosis in a water-tight fashion are two important causes of failure in patency. These technical problems could result from the difficulty in placing microsutures precisely under magnification because of the normal physiological tremor that becomes apparent under magnification.

Robotic systems have recently been introduced in an attempt to reduce the difficulty involved in performing complex laparoscopic urologic procedures. The presence of three-dimensional (3D) magnification and tools with 7 degrees of freedom that are able to duplicate hand movements with high accuracy and imperceptible delay in movement transmission and enhanced dexterity. Conventional surgical manoeuvres are performed at a remote "master" console that precisely translates those movements to robotic "slave" arms.

In the last year, some authors proposed to perform the reversal vasectomy using the da Vinci robotic technology with the aim to eliminate any normal physiologic tremor of the surgeon increasing the technical precision of the suture placement at the anastomosis. In this article, the state-of-the art concerning the application of the robot-assisted reversal vasectomy is reported.

Robotic vasovasostomy

Microscope-assisted vasovasostomy is considered the gold standard treatment for patients who required to restore fertility after a vasectomy. This microsurgical procedure is technically difficult and requires special training considering that the vas is only a few mm in diameter. These technical issues implied that better results were reported by very specialized and well-trained microsurgeons. One potential limit of the microsurgery is correlated with the difficulty to place the anastomotic suture because of the physiological tremor being more evident during a magnificated procedure. In the first years of last decade, general surgery and urology entered the robotic age. After urologic oncology, reversal surgery followed the technological path to robot-assisted procedures.

Robotics may be the surgical adjunct that is needed to overcome the microsurgical challenges of tremor, limited dexterity, miniaturized instrumentation and use of extremely fine suture. Theoretically, using a robot to place the microsutures will make the anastomosis step of the procedure easier. On technical point-of-view, one of the most relevant advantage of the robot is represented by the scaling setting with a reduction gear of 5:1, which allows the surgeon to make large, gross movements at the robot console that are reduced to fine movements at the end of the robot arms. This technical advantage allows us to eliminate any normal physiological tremor of the surgeon. Moreover, the machine allows a degree of precision beyond that possible with human hands. Therefore, robot-assisted reversal vasectomy could be considered as an excellent compromise between optical magnification (inferior to microsurgical approach) and technical precision of the anastomosis by eliminating the physiologic tremor. Obviously, the application of this technique is justified only in centres equiped with the da Vinci robot. In these centres, it is possible to estimate that the reversal vasectomy could be burdened by additional costs of few hundred euro/dollars [14]. Only few studies report the results of robot-assisted vasectomy reversal in experimental animal models and in human.

The first report from Kuang et al. was performed in 2004. In this study the Authors evaluated the feasibility of a robotic assisted vasovasostomy (RAVV) and compared performance measures and adverse haptic events (broken sutures, bent needles or loose stitches) with those of conventional microsurgical assisted vasovasostomy (MAVV) robot for vasovasostomy in human vasectomy tissue ex vivo. The results of this study demonstrated the feasibility of RAVV in this human ex vivo vas model. Specifically, operative time and number of adverse haptic events were higher for RAVV in comparison with MAVV (84 *vs*. 38 minutes, p = 0.01; 2.4 *vs.* 0.0 events, p = 0.03). The number of needle passes required for the 6 full-thickness stitches was similar in both groups. No tremor occurred during RAVV, minimal to moderate tremors occurred during MAVV. Patency of the anastomosis was confirmed in all evaluated cases [16].

In the same year, Schiff et al. reported the first randomized trial comparing standard microsurgical vasovasostomy and vasoepididimostomy to da Vinci robot assisted procedures. an experimental study in which randomized 24 rats to undergo microsurgical multilayer

vasovasostomy or a robotic procedure. In this study the Authors described also the possibility to perform robotically a vasoepididymostomy. The robotic vasovasostomy was significantly faster than the conventional microsurgical technique. Vice versa, considering the vasoepididimostomy no significant

differences were observed in time. Interestingly, the patency rates were 90% after conventional microsurgical techniques and 100% after robotic procedures. However, these differences were not statistically significant. Another critical parameter evaluated was the percentage of sperm granuloma at anastomotic level. Sperm granulomas were found in 70% of microsurgical vasovasostomy anastomoses and 27% of robotic vasovasostomy anastomosis (p = 0.001). No similar differences were found in the vasoepididymostomy group [15].

In the next year, Kuang et al. demonstrated the feasibility of a multilayered robotic-assisted vasovasostomy (RAVV) in a rabbit model. In this experimental study, the surgeon used a 10-0 suture and a two-layer technique to perform both MAVV and RAVV procedures. This study showed that the mean operating time for the total procedure and for the mucosal layer only was longer for RAVV than for MAVV (75 *vs*. 42 minutes, p = 0.03 and 38 *vs*. 23 minutes, p = 0.03, respectively). The needle passes required for the mucosal layer and the number of mucosal and muscularis sutures were similar in both groups (9.5 8.8 passes, p = 0.34; 4 *vs*. 4, p > 0.99; and 7 *vs*. 6.3, p = 0.2, respectively). Unlike MAVV, no tremor was appreciated during RAVV. No adverse haptic events were observed in either group. All anastomoses were patent [17].

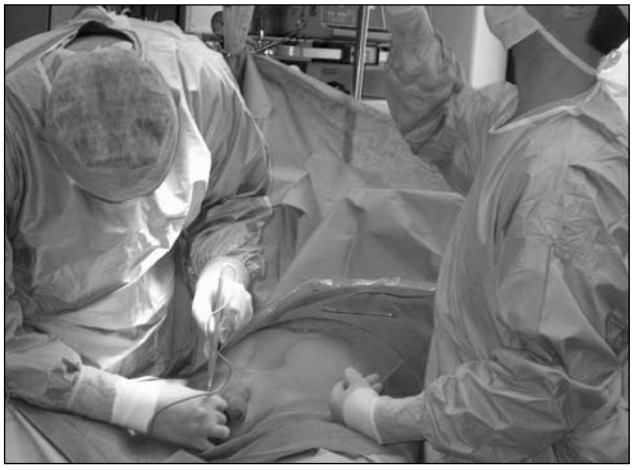
More recently, Parekattil et al. presented a technique and initial results for RAVV in 20 human cases compared with 7 MAVV cases performed by a single fellowship-trained microsurgeon from July 2007 to June 2009. A three-layer 10-0 and 9-0 suture anastomosis was performed. Two-month after surgery all anastomoses were patent in both groups and patients who underwent RAVV reported a slight statistically significant advantage in terms of mean sperm count (54 million *vs*. 11 million, p = 0.04) [8].

Personal Surgical Technique

The patient is in dorsal decubitus with legs together and preferably under general anesthesia, in order to avoid involuntary movements, which would disturb the proper course of the intervention and prolong it needlessly. Antibiotic prophylaxis (cephalosporin) is given. The scrotum is shaved and the skin is disinfected with aqueous Betadine solution.

The surgeon stays to the right of the patient and is assisted by a scrub nurse. A scrotal incision, one inch down from the inguinal ring, is made longitudinally. Alternatively, a median scrotal incision starting from the penoscrotal angle is made over the length of 5 cm (Fig. 1).

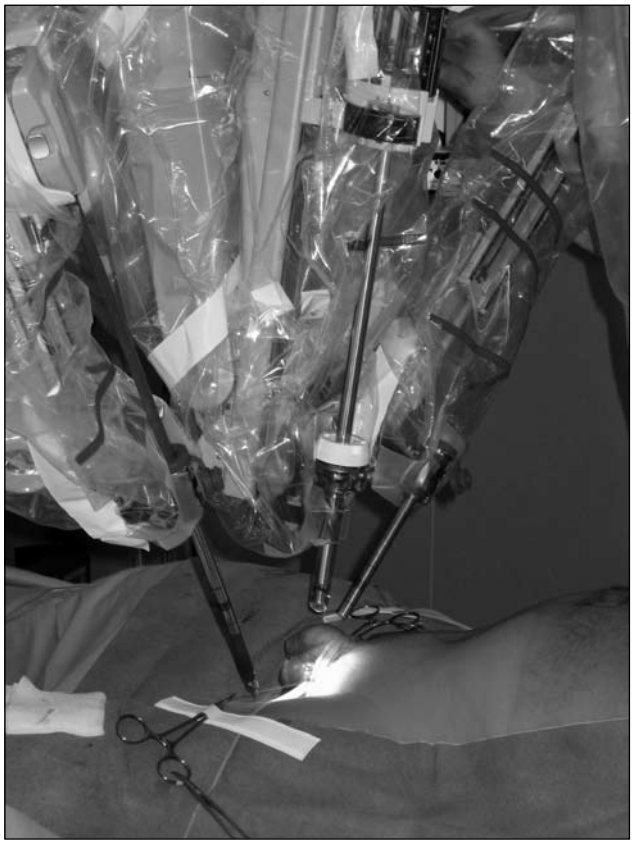
Figure 1.



The vas is delivered from the scrotum. The site of vasectomy is identified and by sharp dissection the proximal and distal ends of the ductus are isolated and fixed, taking care not to injure the blood supply further than it was during the vasectomy. The ends are transsected. The distal end is emptied to check whether a clear fluid or more pus-like fluid comes out of the epididymis. The proximal end is catheterised and 2 cc of saline is injected to control permeability. If the lumen at the proximal end is not open, the end will be cut a few millimetres until an open lumen is reached. The ends are now prepared for the anastomosis. The distal and proximal end are approximated loosely using a 4-0 resorbable suture in the perivasal sheath. One of the Authors prefers to use in this step of the procedure a 7-0 resorbable suture (GAdB).

When both sides have been prepared, the robot is brought into the operating field. The robot must be prepared in advance on the left side of the patient. It is moved forward perpendicularly to the operating field, centered on the vas presented in the Goldstein's microspike approximator. The 0° optic is placed in a 12 mm trocar at an angle of 80°. The arm position should allow movement of a few centimeters to the left of the patient without changing the angle for the second anastomosis. Arms 1 and 2 have 8 mm trocars as in a laparoscopic intervention, but these are used only to support the instruments. They should not be too close to the field, as if they were in the abdominal cavity, and keep a distance of 10 cm between the black band at their tip and the vas. Trocars 1 and 2 hold Black Diamond microforceps. They are placed at an angle of 45° with respect to the optic. Arm 3 is placed on the right. It also carries a 8 mm trocar holding Potts scissors and is placed in front of the camera at an angle of 30° with respect to the patient.

Figure 2.



The first assistant remains in position, the primary surgeon moves to the console. The robot is placed between the legs of the patient. The authors used the Da Vinci Surgical System S (i), Intuitive Surgical, Sunnyvale, California (Fig. 2).

The robotic anastomosis starts with loading two microforceps into the robot. The assistant passes a 8-0 or 9-0 nylon suture into the operating field. We prefer to place three posterior sutures, full thickness, ie. muscularis and mucosa of the vas, tying the sutures when all three are placed. Then the ends are cut. After this posterior anastomosis, five sutures are placed anteriorly, tying each knot when it's placed. To have a better view, the operating field is sprayed with saline using Tissomat (a pressure-device also used by cardiosurgeons to spray Tissucol over their coronary bypasses). At the end of the anastomosis, two or three supporting muscularis sutures are placed. The third arm of the robot can be loaded with scissors to cut the sutures. This option could be considered unnecessary and expensive but reduces the time of surgery. Moreover, there is no need for an assistant and a scrub nurse is sufficient. After this microsurgical part of the operation, the robot is pulled back and the scrotal wounds are closed in two layers using a resorbable 4-0 suture.

Our unpublished data concern 11 robotic-assisted vasovasostomy performed between January 2006 and December 2009 in Aalst, Belgium.

During the post-operative period, we observed only one scrotal hematoma. Six months after surgery all the anastomoses resulted patent. Mean sperm count was 48.6 million per ml after RAVV and 22.8 million/ml after MAVV. In the RAVV group the pregnancy rate was 36%.

Expert commentary

The da Vinci surgical robotic system was rapidly applied in the fields of general, vascular, cardiac, pediatric, gynecologic and urologic surgery. The advantages of this device include improved visibility with a 3-dimensional view, a comfortable and ergonomically superior position during surgery, and increased degrees of freedom of motion of instruments. Moreover, another important advantage is improved stability during suturing as a result of the motion reduction feature in the robot. In the last year, a lot of reports demonstrated as the robot allows surgeons to significantly simplify the more complex reconstructive steps of the laparoscopic procedures. There has been an explosive increase in the number of urologic procedures being attempted using Da Vinci assistance and we believe that robotic technology represents the future of minimally invasive surgery and applications for the robot will expand as more centers report their results. As reported by Fleming et al. in 2004, robot-assisted vasectomy reversal is an attractive alternative to both traditional microsurgical vasovasostomy or vasoepididymostomy for several reasons:

1. the normal physiologic tremor is removed and greater ease and precision of suture placement are possible. While the magnification of the robotic camera (10 to 15x) is not as high as that of the operating microscope (up to 25x), enhanced control with motion reduction compensated for this difference. Data coming from the Literature showed that the precision of suture placement resulted in a more rapid suture placement and watertight anastomosis. Moreover, the robotic technology minimises the differences between placing sutures with the left or right hand, which further facilitates suturing. These concepts find clinical confirmation in the percentages of patent anastomosis and in the percentages of the presence of sperm granuloma;

2. the training period is probably shorter than traditional microscopic techniques and benefits achieved with the surgical robot were acquired with a short learning curve. The learning curve period was considered nonexistent for experienced microsurgeons and probably fewer cases are necessary to become competent also for robotic surgeons without a specific microsurgical training. As reported by Fleming et al., a surgeon without expertise in microsurgical technique should participate in a rat microsurgery course and should have extensive lab animal microsurgery experience performing at least five to eight cases [18]. Vice versa, experienced microsurgeons need to learn to use the robot approximatively 30 minutes in a "dry lab" practicing suturing with a piece of Gore-tex vascular graft and 9-0 nylon suture [14]. However, to be critical, no studies yet demonstrated the exact impact of learning curve for vasectomy reversal procedures;
3. this will allow more surgeons with expertise in robotic surgery to provide quality technical surgical care for their patients. Although data coming from literature are promising, the robot-assisted reversal vasectomy is still in its feasibility phase and most of the available studies were performed in experimental models. Our personal experience confirms the good results reported in Literature in terms of patent anastomoses rate and mean sperm count and pregnancy rate in comparison with the current gold standard treatment represented by microsurgical assisted techniques.

Potential drawbacks to use a robot for reversal of vasectomy are the suboptimal instrumentation available, not designed for microsurgery and the lack of tactile feedback. Another critical issue could be represented by the costs of the procedure. However, it is clear that RAVV must be considered an optional procedure to perform only in centres performing routinely robotic surgery. We believe that the contribution of the robotic surgery to the microsurgical technique has the potential for a more profound impact.

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Relationship between Type A spermatozoa motility in the ejaculate of infertile treated men and the incidence of pregnancy achieved with artificial insemination

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Summary

Objective. Aim of this study was to evaluate the relation between Type A spermatozoa motility in ejaculate of treated infertile men and incidence of pregnancy achieved with artificial insemination.

Materials and methods. 72 infertile males from couples with only male infertility, were included in the study. Seminal fluid was assayed according to the World Health Organization guidelines and semen was prepared through gradient density for insemination. Couples were followed to evaluate pregnancy occurrence. Four categories of Type A spermatozoa were identified: first = 0-10%, second = 11-30%, third = 31-50% and fourth = 51-100%. Data were analyzed using the logistic regression model to estimate the relative risk (RR).

Results. Pregnancy rates in the four Type A spermatozoa categories were: 11.8% (SE = 7.8), 45.0% (SE = 11.1), 26.7% (SE = 11.4) and 30.0% (SE = 10.3). Using the first Type A spermatozoa category as reference, the second category was significantly different in pregnancy incidence (RR = 6.14, p-value = 0.0385), but no difference emerged in the third and fourth category (RR = 2.7, p-value = 0.2924 and RR = 3.2, p-value = 0.1931).

Conclusions. This study shows that having Type A spermatozoa percentage lower than 10% is an unfavorable factor, whereas the highest number of pregnancies occurred with spermatozoa in the 11-30% category. Further percentage increase of Type A spermatozoa did not improve the pregnancy rate. Notwithstanding the little sample size, these data suggest that a significant relation exists between Type A spermatozoa in the ejaculate and pregnancy rate.

Key words
Artificial insemination • Male infertility • Motility • Pregnancy rate

Introduction

Male infertility and its treatment, including techniques for medically assisted procreation, receive growing attention from researchers and specialists in human reproduction [1-3]. A major need, for clinical reasons, is reliable parameters predictive of assisted reproduction success. From this point of view, spermatozoa motility is an important variable.

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According to the World Health Organization (WHO) Manual [4], the initial ejaculate concentration of sperm with progressive rapid and linear motility, or Type A spermatozoa, has been related with *in vivo* pregnancy rate [5] and the proportion of spermatozoa with linear velocity > 22 mm/s distinguishes between fertile and subfertile seminal fluid [6]. In a study published in 1996, the semen characteristics of 672 patients undergoing 950 *in vitro* fertilization cycles and 753 patients undergoing 1,448 intrauterine insemination (IUI) cycles were evaluated. Although no trend was observed in pregnancy incidence in relation to Type A spermatozoa proportion, a significant reduction in *in vitro* fertilization and in the percentage of *in vitro* and IUI pregnancy was seen if Type A spermatozoa were absent using the Percoll technique [7].

Other studies confirm the importance of the presence of spermatozoa with rapid and linear motility in semen [8]. Although ejaculate sperm quality and quantity can be reliable parameters for predicting pregnancy, some authors suggests that spermatozoa motility type is the only valid criterion. They indicate that 40% of motile sperm constitute an adequate cut-off to predict pregnancy incidence, whereas higher values do not significantly increase probability of pregnancy [9]. More recently, the link affinity between sperm and the oocyte membrane and Type A motility were combined to predict oocyte fertilization. When Type A motility was < 5%, fertilization incidence was significantly reduced [10].

Aim of this study was to investigate the relationship between Type A sperm motility in ejaculate from infertile men, pharmacologically or surgically treated, and the frequency of pregnancy obtained with artificial insemination. We aimed to evaluate the existence of a "cut-off value" for Type A sperm motility, predictive of pregnancy.

Materials and methods

Seventy-two consecutive couples with only male infertility were enrolled in this study at the Sterility Center of the Pescia Hospital (Azienda USL 3 of Pistoia, Italy) between January 2002 and January 2009. If the female was older than 40 years or had tubal or endometrial infertility, the couple was excluded. Seventy-two male partners underwent an andrological diagnosis and, when indicated, pharmacological and/or surgical treatment. All data reported in this study refer to ejaculated semen used for insemination.

Male and female characteristics and treatment

For baseline analysis, males were required to undergo two seminal examinations one month apart, and one ejaculation test. These were repeated at every successive check-up following pharmacological or surgical treatment. All examinations of seminal fluid were carried out in the same laboratory of the Center, by the same personnel and according to WHO criteria.

The diagnostic protocol included a scrotal ultrasound examination with ecocolor Doppler for the funicular vessels, endocrine and bacteriological evaluation. A transrectal prostatic-bladder ultrasound and genetic analysis (karyotype and evaluation of chromosome Y microdeletions) were performed in cases of serious asthenoteratozoospermia. Patients with genitourinary tract inflammation with or without positive culture were given antibiotics and antioxidants. Varicocele was treated according to the indications of the *American Urological Association (AUA), American Society for Reproductive Medicine (ASRM)* [11], and idiopathic infertility was treated with antiestrogens or Follicle Stimulating Hormone (FSH) (FSH values < 6.9 IU/L) or not treated.

The diagnostic protocol for the female patients included: hormonal profile during various phases of menstrual cycle, tubal perviousness examination, screening for infections. Patients underwent hormonal therapy with menopausal or recombinant gonadotropins from the day 3 of the menstrual cycle until follicles reached 18-20 mm and hCG (5000-10000 U.I.) were administered to obtain the ovulation. Insemination was performed about 36-38 hours after the hCG.

Semen preparation

Seminal fluid analysis was carried out according to WHO guidelines [4]. On the day of insemination males were required to have no more than two days of abstinence and semen was collected immediately following masturbation. Immediately after liquefaction, semen was centrifuged in a gradient density [Purception, Sage IVF (Cooper Surgical Group), USA]. If the volume was > 2 ml, semen was divided into 2 ml shares and stratified on a gradient consisting of 40% (1 ml) and 80% (1 ml) phases in 15 ml conical centrifuge test tubes. Samples were centrifuged at 300x*g* for 20 min. Afterwards, the obtained pellet was suspended in 2 ml of culture (Sperm Wash, Nidacon International AB, Sweden) and centrifuged at 300x*g* for 7 min. The obtained pellet was suspended again in 4 ml of insemination culture (Sperm Assist, Nidacon International AB, Sweden) ready for insemination. Before and after preparation of semen, data regarding volume, chemical and physical characteristics, concentration and sperm motility were recorded.

Insemination procedure

Insemination (tubal sperm perfusion) was carried out using a two-way catheter (Cervix Adaptor, PBI International). After careful cleaning of the cervix, 4 ml of ejaculate and then 3 ml of air were injected. The device was left in position for 30 min after injection. Female patients received additional therapy in the luteal phase with progesterone until the pregnancy test. If the test was positive therapy was continued until the tenth week of pregnancy, defined as presence of gestational chamber, vitelline membrane and fetal heartbeat.

Statistical analysis

Pearson's χ^2 test [12] was performed to compare categorical variables. Logistic regression model with maximum likelihood fitting was used to estimate RR and their corresponding 95% Confidence Interval (CI) [13].

Results

Type A motility was categorized into four groups: 0-10%, 11-30%, 31-50%, 51-100% (Table I). A different number of insemination cycles in the various motility categories might constitute a confounding factor, thus we performed a preliminary analysis to evaluate a possible relation between number of insemination cycles and category of motility. All couples who had four or more insemination cycles were considered as a single category. These categories corresponded to those obtained on the basis of the percentiles (25, 50, 75, 100). Pearson's χ^2 test gave not significant result (p = 0.6226). We also conducted a logistic regression model analysis considering the level of motility as a possible risk factor for pregnancy. In Table II the Relative Risk (RR), 95% CI values are presented. This analysis was performed including in the model the number of cycles as a confounding variable. Since the results were similar in two cases (p-values = 0.0461, 0.2860, 0.2356 for motility category 2, 3 and 4 respectively), we concluded that the number of cycles was not a confounding factor.

Seventy-two couples enrolled in the study underwent a total of 194 insemination cycles. Each couple underwent one cycle at least. The maximum number of cycles was 6. All females were administered hormonal therapies. Out of 194 cycles, 21 pregnancies were obtained, with a pregnancy rate per couple of 29.2% (Standard Error, SE = 5.4) and a pregnancy rate per cycle of 10.8% (SE = 2.2). The live birth rate was 100%.

Four categories of Type A motility were created for the treated and ejaculated seminal fluid (0-10%, 11-30%, 31-50% and 51-100%).

Table I. Pregnancy rate in Type A motility category.

TYPE A MOTILITY (%)	N. COUPLES	N. CYCLES	N. PREGNANCIES	PREGNANCY RATE% (SE)	
				PER COUPLE	PER CYCLE
0-10	17	41	2	11.8 (7.8)	4.9 (3.4)
11-30	20	57	9	45.0 (11.1)	15.8 (4.8)
31-50	15	35	4	26.7 (11.4)	11.4 (5.4)
51-100	20	61	6	30.0 (10.3)	9.8 (3.8)
Total	72	194	21	29.2 (5.4)	10.8 (2.2)

SE = Standard Error.

Table II. RRs of pregnancy in strata of Type A motility.

TYPE A MOTILITY (%)	PREGNANCY					
	NO		YES			
	N.	(%)*	N.	(%)*	RR (95%CI)	P-VALUE
0-10	15	(88.0)	2	(11.8)	1+	
11-30	11	(55.0)	9	(45.0)	6.13 (1.1-34.2)	0.0385
31-50	11	(73.3)	4	(26.7)	2.79 (0.4-17.6)	0.2924
51-100	14	(70.0)	6	(30.0)	2.95 (0.6-18.6)	0.1931
Total	51	(70.8)	21	(29.2)		

* Row percent; + Reference category.

In the first category, two pregnancies occurred (pregnancy rate per couple 11.8% (SE = 7.8) and per cycle 4.9% (SE = 3.4)). In the second category, there were 9 pregnancies (pregnancy rate per couple 45.0% (SE = 11.1) and per cycle 15.8% (SE = 4.8)), in the third category 4 pregnancies (pregnancy rate per couple 26.7% (SE = 11.4) and per cycle 11.4% (SE = 5.4)), in the fourth 6 pregnancies (pregnancy rate per couple 30.0% (SE = 10.3) and per cycle 9.8% (SE = 3.8)). In 21 couples which became pregnant, we investigated the presence of Type A spermatozoa in the untreated ejaculated seminal fluid. In two cases, Type A sperm was absent (9.5%), whereas in 19 Type A spermatozoa were present before treatment.

In baseline ejaculates, Type A sperm was between 0% and 10% in 52.4% of cases, between 11% and 30% in 28.6%, between 31% and 50% in 14.3%, whereas only 4.7% of ejaculates contained more than 50% of Type A sperm.

Statistical analysis indicated a significant difference in the number of pregnancies between the second motility category and the reference (RR = 6.14; p = 0.0385),whereas third and fourth categories were not significantly different from reference (RR = 2.73; p = 0.2924 and RR = 3.21; p = 0.1931).

Discussion

The aim of this study was to evaluate the potential relation between Type A sperm motility and pregnancy incidence after artificial insemination. In another study [14] we found that pregnancy rate (in the artificial insemination) is statistically higher in males treated compared to untreated, so we can suggest that the medical/surgical treatment may be predictive of assisted fertilization success.

In this study, we observed that a low concentration of Type A sperm (between 0-10%) resulted in a lower percentage of pregnancies per couple and per cycle; when the percentage of Type A sperm increased to 11-30%, both the pregnancy rate per couple and per cycle became four times higher. However, a further increase in sperm of Type A motility (30 to 100%) did not increase the pregnancy rate. Although the presence of Type A spermatozoa is important in successful artificial insemination, our study has a main limitation: the small sample size does not enable us to draw conclusions about the relation between the absence of Type A sperm motility (only one case) and pregnancy. Of the 21 pregnancies achieved in our study, almost 90.5% of males were endowed with spermatozoa of Type A motility prior to treatment. Furthermore, most of these males (52.4%) had between 0-10% of Type A spermatozoa but not more.

Several studies have directed attention to the motility and morphology of spermatozoa used for insemination. While morphology before and after ejaculation is not a parameter which is usually related with pregnancy rate following artificial insemination, when it is united with motility, it can constitute a predictive factor of success of the technique [15-18]. In our study it was not possible to evaluate the relationship of motility and pregnancy rate with sperm morphology since morphological evaluation is usually not carried out on ejaculated sperm used for insemination. Nevertheless, achievement of qualitatively improved motility should be regarded as a goal of primary importance in the management of infertile couples in whom the male is affected by mild or moderate types of infertility.

Conclusion

Baseline values of Type A spermatozoa in the ejaculate of males who are not treated pharmacologically or surgically can be a predictive factor of successful outcome of treatment and insemination.

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Complex congenital pelvic vascular malformations in the male: a rare cause of andrological symptoms. A case report and review of the literature

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Key words
Pelvic vascular malformation • Color Doppler sonography • CT angiography • MRI • Seminal vesiculitis

Summary

We describe a rare case of complex congenital vascular malformation in a young male with a non specific symptomatology characterized by dysuria, pelvic discomfort and pain in the left thigh and left testicle. Moreover we review the data and the syntomatology of the 70 male patients reported in the literature affected with this rare malformation.

Introduction

Complex congenital vascular malformations of the pelvis in male patients are rare [1]. Most men reported unspecific symptoms like pelvic discomfort, pain, impotence or dysuria [2]. Color Doppler imaging is the first choice procedure to make the diagnosis [3-5]; CT angiography can accurately identify the vases involved and the magnetic resonance imaging (MRI) gives accurate anatomical details of the organs interested in the vascular malformation [5].

We describe a rare case of a young male patient observed for a non specific symptomatology. In addition we report a review of 70 male patients reported in literature with congenital pelvic vascular malformations to obtain informations about the most frequent reported symptoms.

Case report

A 28-year-old white male required urological evaluation for a non specific symptomatology characterized by dysuria, pelvic discomfort and pain in the left thigh and left testicle. On the basis of general physical examination and of a real-time sonography of the pelvis it was thought that the patient had a left seminal vesiculitis and it was prescribed antibiotics and anti-inflammatory drugs. During the treatment the symptoms improved but at the end of therapy the symptomatology returned.

When the patient came to our observation we performed a color flow Doppler sonography of the pelvis that showed dilatated vases

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near the left side of the prostate with systolic flow and vases with turbulent blood flow (Fig. 1). These finding were confirmed by the color flow Doppler sonography with “end-fire” transrectal probe that showed more accurately the dilatated vases near the left side of the prostate (Fig. 2). CT angiography showed that there was a complex vascular malformation of the pelvis with multiple macro and micro communications of the hypogastric artery with the venous system (Fig. 3). MRI (Fig. 4) showed that the bladder, rectum and prostate were not involved in the vascular malformation.

Patient underwent a transcatheter embolization of the malformed pelvic vases. The color flow Doppler transrectal sonography performed after the treatment (Fig. 5) showed the absence of flow in the dilatated vases and the patient reported a complete absence of the previous symtomps.

Figure 1. Color flow Doppler sonography of the pelvis. Dilatated vases with systolic blood flow near the left side of the prostate (P).

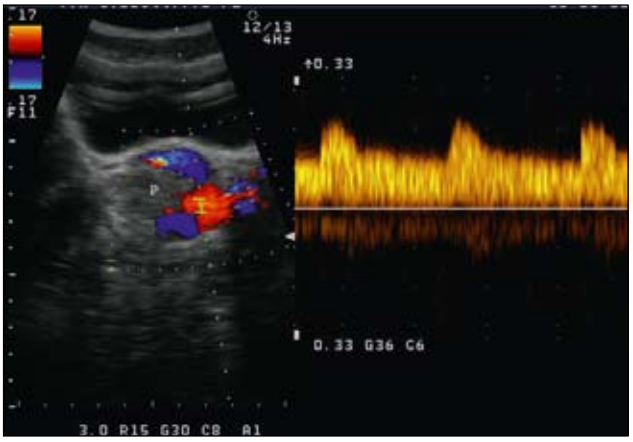


Figure 2. Color flow Doppler sonography with transrectal “end-fire” probe. Dilatated vases with turbulent blood flow near the left side of the prostate (P).

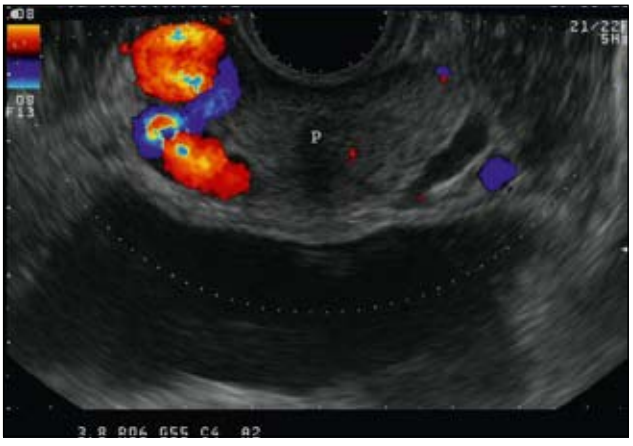


Figure 3. CT angiography. Complex vascular pelvic malformation involved the hypogastric artery with multiple macro and micro communications with the venous system.

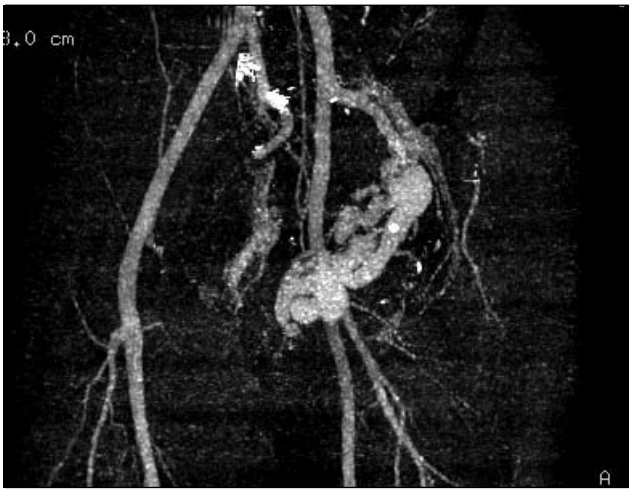


Figure 4. Axial MRI of the pelvis. The dilatated vessels of the complex vascular pelvic malformation (*) do not involve the bladder (B), rectum (R) and prostate (P).

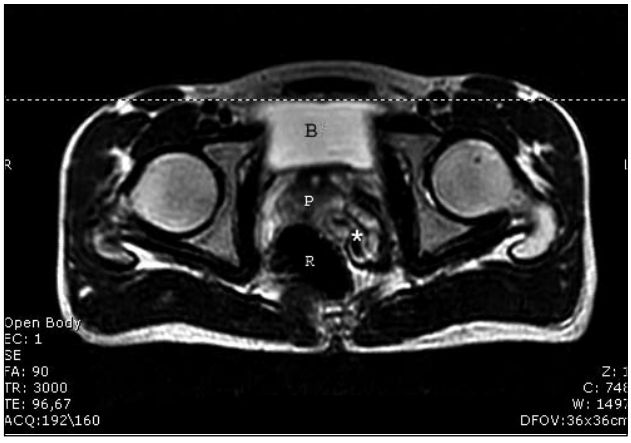
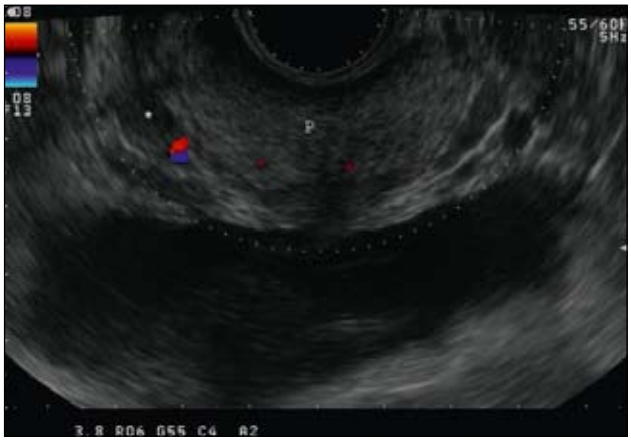


Figure 5. Color flow Doppler sonography with transrectal “end-fire” probe after transcatheter embolization. Absence of flow in the dilatated vases (*) near the prostate (P).



Discussion

Complex congenital pelvic vascular malformations are rare in male patients [1].

It develops as a result of multitudinous embryonic connections between the arterial and the low-resistance venous system [4]. Because of the high flow of blood through inumerable vases a diffuse mass of large-diameter vessels may form in the bowel, bladder and other pelvic soft tissues [6].

No more than 70 patients affected by this rare vascular malformation are described in literature [1 6].

The symptoms more often reported are non-specific such us pain in corrispondence of testicles (62%), buttock and/or thigh (21%), urinary symptoms (such as dysuria and frequency) (20%), postorgams pain (3%), impotence (3%), seminal vesiculitis (21%). The frequency of signs and symtoms related to the mass or to the vascular malformation is: pain (59%), palpable lesion (61%), high-output heart failure (18%), hemorrhage (26%).

It is of note that more than one-fifh of patients are asymptomatic [5 6] and several authors have shown that asymptomatic lesions may be safely followed with no intervention [2].

It has been reported [6] that in two male patients (ages 39 and 40 years) complained of impotence treated with surgical excision of the vascular malformation one regained the ability to attain an erection.

Natali et al. [7] defines the "throbbing buttocks syndrome" a syndrome due to aneurysm of persistent sciatic artery and/or congenital or traumatic arteriovenous fistula of the hypogastric gluteal vessels.

It is interesting to observe that in the two cases described by Mitty et al. [8] the initial clinical impression was that the patients had a subacute seminal vesiculitis and one patient was discharged on antibiotic therapy just like it occourred in our patient.

The natural history of vascular malformations is variable. It can remain asymptomatic for years, when symptoms develop they will usually not resolve without intervention [2 9] and hemorrhage can be fatal [10].

The treatment of congenital pelvic malformations includes surgical devascularization and percutaneous arterial embolization.Surgical treatment of this malformations is notoriously difficult with a high rate of recurrence [2]. Transcatheter embolization therapy seems to be the treatment of choice in the symptomatic patients with pelvic vascular malformations [6].

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