Review Article

Animal models of erectile dysfunction

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ABSTRACT

Animal models have contributed to a great extent to understanding and advancement in the field of sexual medicine. Many current medical and surgical therapies in sexual medicine have been tried based on these animal models. Extensive literature search revealed that the compiled information is limited. In this review, we describe various experimental models of erectile dysfunction (ED) encompassing their procedures, variables of assessment, advantages and disadvantages. The search strategy consisted of review of PubMed based articles. We included original research work and certain review articles available in PubMed database. The search terms used were "ED and experimental models," "ED and nervous stimulation," "ED and cavernous nerve stimulation," "ED and central stimulation," "ED and diabetes mellitus," "ED and ageing," "ED and hypercholesteremia," "ED and Peyronie's disease," "radiation induced ED," "telemetric recording," "ED and mating test" and "ED and non-contact erection test."

Key words: Cavernous nerve stimulation, erection test, intracavernosal pressure, mating test, sexual dysfunction, telemetric recording

INTRODUCTION

Erectile dysfunction (ED) is defined as the persistent inability to achieve or maintain penile erection sufficient for satisfactory sexual performance.[1] The prevalence of ED was estimated to be over 152 million men world-wide in 1995, with predictions that there would be 322 million cases of ED by 2025.[2] It was believed for a long time that there is no organic cause for ED and treatment offered was only in the form of psychosocial counseling. ED is currently treated by medical and non-medical modalities.

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The current knowledge of physiology of erection has been attained from *in vivo* and *in vitro* research on animals. In the second half of the 19th century, it was showed by Eckhard that the pelvic nerve is involved in the erection in dogs.[3] Later on in the year 1968 Lewis *et al.* measured the intracavernous pressure (ICP) in bulls.[4] Eventually, many animal models of different animals such as monkeys, dogs and rabbits were elucidated and used. The use of ICP measurement in smaller animals such as mice and rats also become available commercially, which further aided in study the pathophysiology of ED.[3]

Extensive literature search revealed that compiled information on animal models is limited. The aim of this review article is to provide compiled information on most commonly used experimental models of ED.

The search strategy consisted of review of Pubmed based articles. The search terms used were "ED and experimental models," "ED and nervous stimulation," "ED and cavernous nerve (CN) stimulation," "ED and central stimulation," "ED and diabetes mellitus (DM)," "ED and ageing," "ED and hypercholesteremia," "ED and Peyronie's disease," "radiation induced ED," "telemetric recording," "ED and mating test" and "ED and non-contact erection test." We included original research work and certain review articles, which were freely available on PubMed. Figure 1 depicts various models of ED described in this review article.

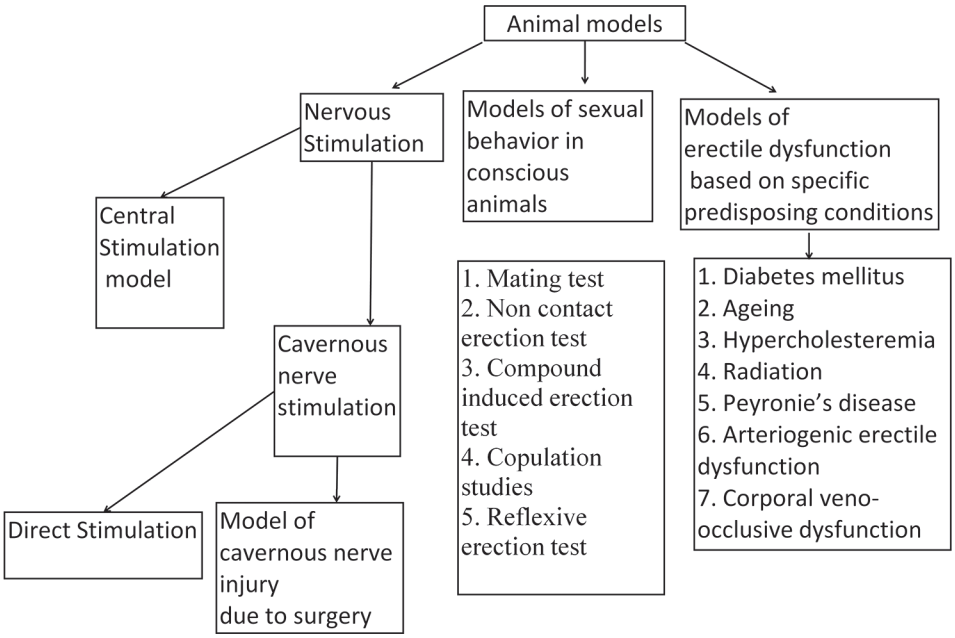

Figure 1: Various models of erectile dysfunction

ANIMAL MODELS

Nerve stimulation models

Stimulation of pelvic plexus or CN results in rise in ICP and submaximal stimulatory voltages are given to generate ICP response. Subsequently, the effect of test drugs are evaluated on this submaximal response.[5] This model of nerve stimulation has been described in rats,[6] dogs[6] and rabbits.[7] A detailed description of different models of nerve stimulation has been summarized in Table 1.

Variables assessed in the models of nerve stimulation are systemic arterial pressure (SAP), ICP, ratio of ICP to SAP and detumescense time which is the time from termination of electrical stimulation until the maintenance of a fixed ICP.[5] The ICP/SAP ratio points towards the degree of relaxation of corpora cavernosa (CC) and deep arteries in the penis.[8] In general, agents that increase the SAP, causes an associated decrease in the ICP/SAP ratio. This is because an increase in SAP results in a corresponding narrowing of the CC.[9] As a corollary, agents inducing a decrease in SAP, increases the ICP/SAP ratio due to the relaxation of the CC. One of the basic advantages of the nerve stimulation model is that the changes in both the systemic and penile vasculature can be recorded simultaneously.[5] Furthermore, even minor changes in the erectile response can be assessed by this method. However, certain drawbacks like the need to anesthetize the animal which might influence the physiology and pharmacological response of erection are associated with this method.[10] Hence, telemetric devices which can record ICP and SAP even in non-anesthetized animals have been developed.

Telemetric recording

A fixed range of ICP and SAP can be recorded by these telemetric devices; also certain telemetric devices can be surgically implanted. Rats are anesthetized and placed on a homoeothermic blanket. The catheter of the telemetric device is placed in the proximal shaft of the right CC along-with the pressure transducer which is kept subcutaneously in the abdominal wall. The SAP is measured by placing the catheter tip in the aorta about 10 mm rostral to the bifurcation of the aorta with the orientation toward the heart. Rats are tested after 7 days. It has been found that there is no variation in the increased ICP recorded by the pelvic nerve stimulation method and through the telemetric method and it does not interfere with the physiologic process of penile erection, nor does it alter the animal behavior related to erection.[11]

Model of post-surgical ED due to CN injury

CN injury post radical pelvic surgery leading to impairment in erectile function occurs even though there have been advances in surgical techniques.[12] Hence, there is a need for a model mimicking this clinical situation.

The model first described was by Quinlan *et al*.[13] Rats were separated into three groups. First was the sham control group, in which only exploration of the pelvis was done, the second was the nerve ablation group in which 5 mm of the CN was removed and the third group was the graft group in which bilateral 5 mm excision of the CN was performed and it was replaced by a graft of the genito-femoral nerve. At 1 month post-surgery animals were subjected to mating tests to determine potency.[13]

Table 1: Models of erectile dysfunction based on nerve stimulation

Animal	Type and weight of animal	Anesthetic technique	Cavernous nerve stimulation	Measurement of ICP	Measurement of SAP
Rat	Male Sprague-Dawley rats weighing 200-250 g	Intraperitoneal injection of urethane (l. 5 g/kg in sterile saline)	1 ms pulses of 6 V at 12 Hz for 1 min. These stimulation parameters, induce a consistent ICP increase to 40-60% of the mean arterial pressure levels, thought suitable for assessing experimental manipulations. At least 10 min are allowed to elapse between successive stimulations	The penis is desheathed and the CC are exposed. A 25-gauge stainless steel needle is inserted into one CC to record ICP (mmHg). The needle is attached to a catheter filled with heparinized saline (25 IU/ml). Catheters are connected to pressure transducers	Catheter filled with heparinized saline (25 IU/ml) is placed into the carotid artery to record arterial BP (mmHg)
Rabbit	Male New Zealand white rabbits (3.5-4.0 kg)	Intramuscular ketamine (35 mg/kg) and xylazine (5 mg/kg) and animal is placed in the supine position. Anesthesia is maintained as needed with IV sodium pentobarbital 50 mg/mL)	10-V pulse amplitude, 0.8-ms pulse width, and suboptimal frequencies (2.5 or 6 Hz). The interval between stimulations was 3-5 min	A 23-gauge needle, filled with 4 U/mL heparin solution and connected to PE-50 tubing is inserted into the left CC for pressure recording. All pressure measurements are recorded by means of grass PT-300 pressure transducers connected signal conditioner modules and a grass 7400 physiological re-corder	A 3 cm midline neck incision is fashioned to access the carotid artery. A 20-gauge angiocatheter is inserted into the carotid artery and connected to a PT-300 pressure transducer
Dog	Male beagle dogs weighing 10-12 kg	Pentobarbital sodium (nembutal, 35 mg/kg IV for induction and 4 mg/kg/h for maintenance)	Electrical stimulation with trains of pulses of 10 V, 15 Hz, 8 ms for 25 s are delivered to analyze the effects of drugs on ICP rises elicited by nerve stimulation	20-gauge needle placed into the left or right corpus cavernosum. The needle is attached to a catheter filled with heparinized saline (25 IU/ml). Pressure signal is triggered by carrier amplifiers on a multichannel polygraph	BP is monitored via a pressure transducer introduced into the aortic arch through the right common carotid artery

ICP=Intracavernous pressure, CC=Corpora cavernosa, BP=Blood pressure, IV=Intravenous, SAP=Systemic arterial pressure, PT=Pressure transducer, PE=Polyethylene

The model subsequently described simulates ED post-surgery and can be used for the evaluation of neuroprotective agents, which might help in nerve regeneration and facilitate functional recovery of penile erection. After anaesthetizing a rat, a lower midline incision was made, pelvic organs pushed up in the epigastrium and dorsal lobes of prostate was exposed. The CN was identified by the surgical microscope. The CN was then hooked with bipolar electrodes and the identity of CN was confirmed by the occurrence of tumescence after its electro-stimulation. CN was then transected in unilateral or bilateral manner. Studies have elucidated neuronal nitric oxide synthase (nNOS) contained in nerve fiber regenerating in cavernosum following unilateral but not in bilateral CN injury.[14] Hence the two types of CN injury are essentially different. CN can also be ablated or a crush injury induced by pressure application about 1 mm below the major pelvic ganglion unilaterally or bilaterally.[15,16] This results in loss of erectile function in early post-operative period. Variable recorded in the model of CN injury is ICP. Under anesthesia, a bipolar stainless steel electrode is placed around the CN and stimulated (4 V, 16 Hz, 5 ms) for 1 min, two to three times allowing 15 min intervals between stimulations and ICP is monitored and recorded.[15] Penile erection can be also induced by injecting apomorphine 150 μg/kg. The number of penile erections were observed and counted over a period of 30 min. An erection is defined as when emergence of an engorged glans penis and distal shaft is noted. Ease of identification of the CN, low cost of the model and easy availability of experimental animals are the advantages associated with this model.[16]

Central stimulation models

The central and the peripheral autonomic nervous system is integrated in the paraventricular nucleus (PVN) of the hypothalamus. Penile erections can occur on activation of these neurons by dopamine and its agonists, excitatory amino acids (N-methyl-D-aspartic acid) or oxytocin or by electrical stimulation.[17] Stereotactic chemical or electrical stimulation of the hypothalamic PVN or medial pre-optic area (MPOA) can be utilized for assessing penile erection.[5] Unilateral stainless steel guide cannula ending 1 mm above the left MPOA (from bregma" anterior-posterior = +2.4, medial-lateral = +0.2, dorsal-ventral = –7.0, incisor bar = +5). The guide cannulae were constructed of 23-gauge thin-wall stainless steel tubing (outer diameter = 0.6 mm, inner

diameter = 0.4 mm).[18] Penile erection is recorded after giving electric or chemical stimulation.[19] This model is of great value to elicit the role of various neurotransmitters involved in penile erection.

Models based on sexual behavior in conscious animals

There is a great variation in the reproductive behavior amongst different species and hence before studying the effect of pharmacological interventions on reproductive behaviors, its physical and temporal facets need to be objectively and robustly summarized. Furthermore, due consideration should be given to whether the animal is diurnal, nocturnal or crepuscular since this information can be used for the determining the duration and cycle of light cycle, which might also influence reproductive behavior. In summary, the environmental conditions conducive for reproductive behavior should be given due consideration before planning the execution of models of sexual behavior in conscious animals.[5]

Mating test

Ovariectomized female rats screened with sexually experienced male rats and those with good sexual receptivity (lordosis on mounting and no rejection behavior) are selected for this test. For the test, a male rat is placed in an observation chamber for 5 min for adaptation. Then female is placed in the chamber, which is made receptive by sequential subcutaneous injections of 30 μg estradiol benzoate and 500 μg progesterone 24-48 h and 4 h and earlier, respectively.[20] Number of mounts, intromissions and ejaculations are recorded and scored. A mount is defined when a male rat mounts the female from the rear and grasps her flanks with his front feet. An intromission is defined when a male achieves vaginal penetration. Ejaculation is identified by a prolonged intromission often with deeper thrusting and a slower withdrawing "crucifix" posture post-ejaculation. The test is stopped if no intromissions occur within 15 min. Ejaculations are confirmed by the presence of a semen plug in the female's vagina.[5] However the basic problem with this test is that the parameters evaluated are subjective. However this test can be made more robust on including objective parameters like ICP and SAP. ICP peaks are compared with observations related to mounts and intromissions are coupled with SAP.

Non-contact erection tests

In this test an observational chamber which is divided into half by a barrier is used. Males are placed in one side of the observational chamber and a receptive female is placed in the opposite side. The barrier avoids direct contact between animals on either side, but allows auditory, visual and olfactory stimuli. The behavioral observations are performed for 30 min. An erections is counted when the penis emerges from the penile sheath, accompanied by head and hip flexion.[11] Parameters recorded are total number of penile erections and the time to first erection.[20] Severe impairment in non-contact erection is seen on lesions in the bed nucleus of stria terminalis.[21] Use of subjective parameters is a major drawback of this model. However, this can be overcome by making the parameter objective by inclusion of ICP measurements. This model simulates psychogenic sexual motivation or libido.[20]

Compound-induced erections

This model is used to identify drugs which initiate penile erections. Penile erections are induced in male rats by injecting drugs to them in the absence of female rats. However, one should note that spontaneous penile erections can oocur in animals even by simple actions like handling and hence one should include time matched controls.[5]

Copulation studies

For copulation studies end points like intromission ratio (intromission frequency/[intromission frequency + mount frequency]) are employed as surrogate indicators of erectile function. Numerous factors such as locomotion, motivation and partner animal might manipulate the copulatory behavior of the animals and hence in lieu of presence of these many variables it can be challenging to attribute the effect of a pharmacological intervention on a penile erection. Any differences that are observed between the pharmacological interventions should be confirmed with more robust variables like ICP measurements.[5]

Reflexive erection tests

To perform this test, male rats are restrained on their back and the prepucial sheath is tonically retracted with a metal loop. Lengthening of the penile body, glans engorgement, cups (intense glans erection with flaring of glans extremity) and flips (dorsiflexions of penile body) are the reflex responses which are observed and counted. The observational period is of 15 min, starting from the first reflex response. One can also monitor the ICP simultaneously. Generally the peaks of ICP are seen during reflex erection, which cease to occur after the pelvic nerve is cut bilaterally. This model gives an insight in the physiology of erections that the spinal autonomic nuclei (parasympathetic activity), are originators of reflexive erections.[11]

Models of male ED based on specific predisposing conditions

Diabetic associated ED model

This model simulates changes in diabetes. Rats are injected intraperitoneally with streptozotocin (STZ) 65 mg/kg.[22] Rats achieving a blood glucose level of 300 mg/dL 72 h later are selected for the study. The increased latency for erection, a slower phase of detumescence and lower ICP is seen in these animals 3-6 months later. The penile erection in the model of ED in diabetes induced by STZ is normal to nitroglycerine and papaverine; however, this same response is inadequate to nerve stimulation.[23] Another study done in diabetic rats pointed that in diabetes, even though the ICP does not change during erection the rate at

which the sympathetic tone is re-established is decreased resulting in the ICP pressure to fall even more slowly during detumescence.[24] Another model of ED in mice is described by Xie *et al*. In this model, DM is induced in C57BL6 mice by feeding them a high fat diet (45% of total calorie intake) for a period of 22 weeks.[25] Another model for studying the pathophysiology of ED has been described by Akingba and Burnett who used alloxan (single dose 140 mg/kg intraperitoneally) to induce diabetes in adult male Sprague-Dawley rats.[26] Penile reflex test is used to assess ED. Early and late changes are assessed by sacrificing the rats at 4-5 weeks and 10-11 weeks respectively. Other type 2 diabetic rat models for studying ED are obese Zucker diabetic fatty, the BBZ/WOR rat and the Otsuka Long-Evans Tokushima Fatty rat. Mouse models available are, high fat diet fed, Tsumara Suzuki Obese Diabetes, KK, New Zealand obese, ob/ob and db/db mice.[27]

nNOS and endothelial nitric oxide synthase (eNOS) assay, western immunoblotting and immunohistochemistry examinations are conducted on the penile tissues of these animals. Although nNOS expression and eNOS activity reduce early on, even before ED becomes symptomatics, nNOS and eNOS activities and expressions both decrease simultaneously with symptomatic ED. Corporal tissue is dissected and studied for: (i) Endothelium-dependent and endothelium-independent vasoreactivity. (ii) Endothelial and smooth muscle cell content by immunohistochemistry. (iii) NOS expression by nicotinamide adenine dinucleotide phosphate-diaphorase staining. (iv) Apoptosis by terminal deoxynucleotidyl transferase biotin-d-UTP nick-end labeling (TUNEL) staining.[25] In diabetic mice, apoptosis is found to be higher while CD31 content of endothelial cell, ratio of the smooth muscle to collagen and reactivity of the blood vessels is lower as compared to normal. This points that ED in diabetes is mainly due to endothelial dysfunction which might lead to impaired ability of the endothelium in maintaining vasodilatation and vascular homeostasis.[25] The non-adrenergic-non-cholinergic nerve signaling and penile endothelial functions are affected in type I DM while only endothelial dysfunction occurs in type II DM. Also there is an increased sensitivity of cavernosal tissue to contractile stimuli resulting in a veno-occlusive disorder in type II DM.[28] Such changes are seen in animals models of ED also.[25] This model thus serves as a valuable tool for advancing our understanding of the role DM plays in the pathogenesis of ED.

Aging model

Factors such as increase in the penile vascular tone, decline in acetylcholine mediated relaxation and endothelial dysfunction contribute to the age related ED.[29] Other factors that play an important role in aging induced ED are reactive oxygen species and low testosterone levels.[30,31] Male brown Norway rats (25 month-old), Sprague-Dawley (9-10 months), mice (22-26 months) or rabbits (20 month) are used in age-related ED models.[29,32-34] The drugs are injected directly in CC and ICP is recorded.

Hypercholesterolemic models

Atherosclerotic changes in penile arteries due to hypercholesterolemia partial occlude blood flow further compounding the impairment of endothelium mediated relaxation of blood vessels. Further hypercholesteremia results in alteration in the formation of arachidonate and cyclooxygenase products and endothelium independent cyclic guanosine monophosphate (cGMP) dependent corporal smooth muscle relaxation. Adult male New Zealand white rabbits are used for development of hypercholesterolemia induced ED model. Rabbits are divided into two groups; one was given normal diet and the other diet containing 2% cholesterol for 12-14 weeks. 0.2 mg/kg of sodium nitroprusside is administered which is followed immediately by test drug or saline to the respective groups in the lateral ear vein.[35] A sliding caliper is used to measure the length of the exposed penile mucosa at different time intervals for up to 5 h and the difference between the test and control is compared.[36]

A mouse model of hypercholesterolemia induced ED was described by Xie *et al*. Apolipoprotein E knockout (ApoE –/–) mice are fed a 1.25% cholesterol diet for 2, 4, 8 and 12 weeks while a group of ApoE –/– and wild-type Bl-6 mice are fed a normal diet.[37] Vasoreactivity, histology and protein studies of the corporal tissues of these animals were conducted after sacrificing them at 22 weeks of age and dose response curve are generated.[37] Immunohistochemistry staining or Masson staining is used to determine the endothelial and smooth muscle contents and deriving the smooth muscle/collagen ratio. cGMP level is assessed by enzyme immunoassay and western blot test is used to determine phosphorylated eNOS/total eNOS, nNOS and cyclic GMP-dependent kinase protein.[37]

Behr-Roussel *et al*. developed atherosclerosis-associated ED in the ApoE –/– mice.[38] 26, 32 and 38 week ApoE –/– mice were used for the experiment. Erectile function was evaluated by recording the rise in ICP following the CN electrical stimulation. The lesions of atherosclerosis were examined using planimetry in oil red O-stained aortas. It was found that impaired ED persisted in the 32 and 38 week ApoE –/– mice.[38]

The acute effects of hypercholesterolemic diet on erectile responses was studied by Demir *et al*. Sprague-Dawley rats are fed 1% cholesterol-enriched diet daily for 2 weeks for this purpose. The effects of this diet on ED was evaluated by recording the ICP, SAP and detumescence time following CN stimulation.[39]

Radiation induced ED

Radiation is delivered to rats anesthetized using pentobarbital to the prostatic region. Care is taken to cover the testes with

lead shield to avoid radiation induced damage to fertility. A beam of 250 kVp radiation is administered at a dose rate of 424 cGy/min. ED in rats is evaluated by recording the ICP following electrical stimulation at day 1, 10 days, 20 days and 1 month.[40] Another model described by Kimura *et al.* mimics the radiation induced ED after prostate confined modern radiotherapy.[41] Adult male rats aged 10-12 weeks old are used in this experiment and delivered a single 20-Gy fraction of radiation with due care taken to protect the penile bulb, shaft and testes from treatment fields. ICP is recorded at 2, 4 and 9 weeks following radiotherapy and it usually shows a decline from the 4th week onwards. The CC perfusion is evaluated using the Hoechst florescent stain.[41]

Peyronie's disease like model of ED

Surgical trauma and transforming growth factor-beta 1 (TGF-β1) are implicated in Peyronie's disease seen in humans.[42] El-Sakka *et al.* first developed a rat model of ED due TGF-β1 injection.[42] Later Bivalacqua *et al.* used the model where in adult male CD rats are used. In one group, TGF-β1 (0.5 μg) is injected into the tunica albuginea while in another group surgical injury to the tunica albuginea is induced and both the groups are assessed for erectile function by CN stimulation and chemically by acetycholine. It was found that ED occurs in the TGF-β1-injected and surgical-injury rats after 6 weeks.[43]

Fibrin has been hypothesized to be involved in the pathophysiology of development of Peyronie's disease. Davila *et al.* developed a rat model of Peyronie's disease by injecting fibrin in the tunica albuginea of rat penis.[44] Fibrin is injected locally in the tunica albuginea of rats. Different sets of rats are then sacrificed at 1, 3 and 6 weeks after the injection. These tissue sections are stained with Masson trichrome, Verhoeff's stain and Hart's stain for quantitative analysis of collagen, fibrin and elastin respectively. Although TGF-β1, inducible nitric oxide synthase, hemeoxygenase 1 (HO1), alpha-smooth muscle actin (ASMA), apoptosis (TUNEL) and plasminogen activator inhibitor (PAI) are assessed by immunostaining. Electron microscopy is used to assess collagen fiber organization. 1 week following injection of fibrin, only oedema was present; at 3 weeks, the characteristic fibrotic Peyronie disease like plaque develops. At the same 3 weeks time these rats show increase in the expression of markers of fibrosis like HO1 (reactive oxygen species), ASMA (presence of myofibroblasts), apoptosis and PAI (inhibitor of fibrinolysis).[44] An advantage of these models is, they have exhibited that this tissue is under chronic fibrotic and antifibrotic turnover and have made clear the role of myofibroblasts, microtrauma and oxidative stress in plaque development and these models have displayed to interaction of fibrinolytic and collagenolytic sytems and their inhibitors and also have identified native antifibrotic process involving inducible NOS. Furthermore, these models have helped in detection of stem cells in tunica albuginea, which might have a potential role in fibrosis and ossification.[45]

Arteriogenic ED

El-Sakka *et al.* first developed a rat model of arteriogenic ED.[46] These investigators induced ED by ligating the internal iliac arteries bilaterally. As a modification of this method balloon endothelial injury of the iliac arteries was performed in rabbits. The parameters assessed are the ICP and the and intracavernosal blood flow. The early phase of arteriogenic ED is marked by decreased in-flow and perfusion pressure. Further, the later stage is exemplified by hemodynamic dysregulation and functional impairment associated with down regulation of endothelial and neural NOS.[47]

Corporal veno-occlusive dysfunction

Aged rat is an appropriate model of ED due to CVOD. Davila *et al.* showed venous leakage occurred through the deep dorsal vein in aged rats was further confirmed by occurrence of penile engorgement by application of deep dorsal vein ligation.[48]

CONCLUSION

As can be seen in the assemblage of animal models collated in this review one can understand that rat is the most common laboratory animal used for developing models of ED. Simple telemetric devices, which record sexual responses have facilitated objective assessment in animal models. Use of conscious animal models, which provide natural contexts for sexual physiology and avoid pharmacological interference associated with anesthetics is an important advance in the field of research on drugs for ED.

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