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Biological and clinical research on male reproduction and fertility regulation

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Abstract. Biological and clinical research on male reproduction and fertility regulation carried at the National Institute of Health and Family Welfare over the past 17 years has been highlighted in this review. Areas of research covered pertains to hormones in relation to sperm maturation and transport; fertilizing ability of spermatozoa under different experimental conditions; agents producing functional sterility; seasonal variations in primate reproduction; male infertility including semen biochemistry, differential diagnosis between obstructive and non-obstructive azoospermia and hormone therapy; vasectomy, reversible vasocclusion and vasanastomosis; and the use of cyproterone acetate and testosterone enanthate in male rhesus monkey and human volunteers for reversible contraception.

Keywords. Male reproduction; male sterility; male contraception; sperm maturation; sperm transport; fertilizing ability; functional sterility; primate reproduction; semen biochemistry; vasectomy; vasocclusion; vasanastomosis; cyproterone acetate; testosterone enanthate.

Introduction

The need for fertility control through voluntary efforts can hardly be over-emphasised. The currently estimated population is about 720 millions and there are about 120 million couples in the reproductive age group. For achieving a target of net reproduction rate of unity by the year 2000 A.D., 60 % of the eligible couples have to be effectively protected through contraceptive practices. At the present moment it is estimated that about 29 % of these eligible couples are being protected through contraceptive practice. Therefore, the enormity of the problem and the magnitude of the task can be well appreciated.

Barren marriages bring personal miseries and social stigma in almost all cultures in the world. Its repercussions in the individual families are often so great that development of better tools for its investigation and management assumes major significance. In India, reliable information is not available about the incidence and various causes of infertility and sub-fertility. Even taking a moderate estimate of the incidence at 10%, there will be more than 12 million couples with the problem of

Abbreviations used: GPC, Glycerylphosphorylcholine; CPA, cyproterone acetate; TE, testosterone enanthate.

primary and secondary infertility in the country, and consequently more than 24 million people are directly unhappy because of this problem. Therefore, research on various aspects of infertility for improvement in diagnosis and management of infertility is also important.

Over the past 17 years, systematic studies in several areas of reproduction and fertility regulation in the male and female have been carried out at the Department of Reproductive Biomedicine of the National Institute of Health and Family Welfare (Roy *et al.,* 1976). This review will be restricted to biological and clinical research carried out on male reproduction and fertility regulation.

Biological studies

Hormones in relation to sperm maturation and transport

The activity of the male accessory sex organs as well as the acquiring of motility and fertilising ability of spermatozoa in the epididymis and their maintenance in the vas deferens are androgen dependent. Accordingly, some investigators attempted to produce functional sterility in rats by creating differential androgen deprivation after administration of microdoses of an anti-androgen, cyproterone acetate. The development of reversible methods of functional sterility in the male was an attractive proposition. However, to develop a rational basis for such an approach further work was necessary to determine the role of androgen and other sex steroids in the development and maintenance of sperm maturation in the epididymis and vas deferens. Studies were undertaken for determining the hormonal requirements for the motility and transport of spermatozoa in the epididymis and vas deferens, as well as for better understanding of the physiological functions of these organs.

Hormonal responses of the spermatozoa and accessory sex organs: Following castration of adult rats, a daily dose of 2 μg of testosterone maintained the motility of spermatozoa for 14 days, a dose of 1 μg/day maintained for two days and 4 μg/day for 5 days. The epididymis was found to be more sensitive to testosterone than the seminal vesicles or ventral prostate (Das *et al.,* 1973).

The effects of estradiol-17β and testosterone on the motility pattern and transport of spermatozoa and the weight of accessory genital organs of castrated rats were investigated after 7, 14, 21, 28 and 35 days of treatment. Estradiol-17β could maintain the normal motility pattern of spermatozoa in the terminal segment of the epididymis and vas deferens, and increased the weight of the vas deferens and seminal vesicles only in the group receiving treatment for 7 days. Testosterone either alone or with estradiol-17β maintained sperm motility for 14 days after castration. While testosterone retarded sperm transport in the male tract, estradiol-17β hastened the process (Bandopadhyaya *et al.,* 1974; Das *et al.,* 1976).

Uptake of radioactive testosterone and estradiol by the reproductive organs of male rats: The biological actions of sex steroids are exerted only after their binding with the tissue receptors. The differential uptake of radio-labelled testosterone and estradiol would possibly indicate the relative importance of these steroids for the regulation of

specific biological properties of different segments of the epididymis and other sex accessories. Prevention or alteration of such uptake by various agents might be indicative of possible interference with the control of biological actions and thus, screening of agents with such property might lead to the development of suitable antifertility agent. Accordingly, the uptake of estradiol-17β-6, 7-^{3}H and testosterone-7-^{3}H by various tissues of castrated male rats was studied. The uptake of testosterone-7-^{3}H was more in the proximal and middle segments than that in the terminal segment of the epididymis or in the vas deferens. Treatment with norethindrone reduced the uptake in all the accessory genital organs. 17α-Hydroxy progesterone caproate had similar effect, but of a lesser magnitude.

The uptake of estradiol-17β-6, 7-^{3} H was more in vas deferens than in epididymis. Cold testosterone reduced the uptake of estradiol-17β-6, 7-^{3}H in the vas deferens and epididymis. Following castration the uptake of radio-estradiol was increased in the epididymis and seminal vesicles of rats but not in other organs, whereas administration of 17α-hydroxy progesterone caproate reduced the uptake of labelled estradiol-17β in all the accessory genital organs (Das *et al.,* 1976; Kumari *et al.,* 1976).

Metabolic studies on the epididymis, epididymal spermatozoa and vas deferens in rats: In order to understand certain aspects of the metabolic interrelationship between the epididymis, epididymal spermatozoa and the vas deferens, the glycogen content of the epididymis and the vas deferens and the cytochemical distribution of glycogen in the epididymis and its luminal spermatozoa were studied in normal, castrated and castrated plus low-dose androgen treated rats. The glycogen content of the epididymis as well as the vas deferens was reduced following castration. While daily intra-muscular administration of 1 μg of testosterone for 7 days increased the glycogen content of the vas deferens in castrated rats, a daily dose of 2 μg of testosterone was necessary for its increase in the epididymis. In further studies it was noted that after 7 days of castration, there was a reduction in the content of glycogen, glucose and lactic acid in the epididymis, but not of pyruvic acid. In the vas deferens, the content of glycogen only was reduced following castration. Administration of testosterone to castrated rats in daily doses of 1–16 μg/100 g body weight for 7 days caused an increase in the content of glycogen in all the segments of the epididymis and vas deferens, of glucose in the terminal segment of epididymis but not in the initial and middle segments. The glycogen and glucose content of the vas deferens was more sensitive to testosterone treatment than those in the epididymis (Bandopadhyaya and Das, 1982).

From these results it appears that the carbohydrate metabolism of the epididymis and vas deferens are regulated in a differential manner by androgen. This may have some physiological significance since in the epididymis the spermatozoa undergo the process of maturation, whereas in the vas deferens the matured spermatozoa are stored and their fertilizing ability is maintained.

The binding of antisera to sex steroids on human and rhesus monkey spermatozoa: Sex Steroids bind with the tissue receptors in epididymis and other accessory genital organs and thereby regulate the physiological functions of these organs. In the epididymis, the sperms undergo maturation, the process being under the control of sex steroids. However, the steroids do bind directly on the surface of the spermatozoa which may have important physiological significance. Accordingly, the presence of different sex

Steroids on the surface of ejaculated spermatozoa was studied by indirect immunofluorescence studies. There was no difference in the binding pattern of antisera to steroid hormones on the human and monkey spermatozoa. Assuming the intensity of fluorescence being proportional to the concentration of the hormone, the concentration of testosterone on the acrosomal and post-acrosomal regions were higher than that of estradiol and progesterone (Allag *et al.,* 1983).

Utilizing the same technique changes in the binding sites of testosterone, progesterone and estradiol-17β on the surface of rhesus monkey spermatozoa during their transit from testis to vas deferens were studied (Allag, I. S., Warikoo, P. K., Das, R. P. and Roy, S., unpublished results). The testicular spermatozoa were devoid of any steroid bound on their surface. Testosterone was localized at the mid-piece and principal-piece of spermatozoa collected from caput epididymis; this localization gradually shifted towards the acrosome region during transit through the epididymis. Progesterone was localized at the post-acrosome and mid-piece regions of spermatozoa without exhibiting any shift during transit. Estradiol-17β was bound at the mid-piece and principal-piece regions of epididymal spermatozoa, but the spermatozoa obtained from the vas deferns had additional estradiol binding sites at post-acrosome region. These results provided the first evidence that testosterone and estradiol-17β binding on the surface of monkey spermatozoa change their locations during transport in the male genital tract, which might be linked with the physiological process of sperm maturation (Allag, I. S., Warikoo, P. K., Das, R. P. and Roy, S., unpublished results).

Role of vas deferens: Hormonal and biochemical studies carried out in this Department have indicated that the vas deferens is not merely a tube for the passive transport of spermatozoa, but it is a dynamic organ having important role in the maintenance of functional integrity of the spermatozoa (Kumari *et al.,* 1979, 1980).

The significance of the findings of the hormonal studies carried out is not yet quite clear. Nevertheless, these have provided considerable insight into our understanding of the hormonal regulation of the motility, fertilizing ability and transport of the spermatozoa. These have practical implications. Further elucidation of these leads is in progress.

Studies on the fertilizing ability of spermatozoa under different experimental conditions

There is a lack of reliable criteria for testing the fertilizing ability of the spermatozoa. In order to get a fairly good assessment, a number of complementary tests have been established in this laboratory. These include the following:

(a) *In vivo* fertilization tests for animal spermatozoa.
(b) *In vitro* fertilization tests for animal and human spermatozoa.
(c) *In vitro* cervical mucus penetration tests.
(d) Estimation of the activity of spermatozoal enzymes connected with acrosomal action and sperm motility.

Establishment of these facilities has not only provided means for screening and assessing agents or drugs with potential action, but also has helped in clinical assessment of infertile couples.

In vivo fertilization test was being carried out to determine whether there is any difference between the duration of maintenance of the motility *vis-a-vis* the fertilizing ability of spermatozoa obtained from the cauda epididymis of castrated rats. It was demonstrated that administration of testosterone in doses of 25–100 μg could maintain the fertilizing ability of the spermatozoa for 9–11 days in castrated rats, and the fertilizing ability of the spermatozoa was lost earlier than their motility (Warikoo and Das, 1983a).

The test was also carried out to determine the fertilizing ability of spermatozoa obtained from rats treated with the progestogen STS 557 as described later (Warikoo and Das, 1983b).

The capacity of human and monkey spermatozoa to fertilize the zona-free hamster eggs was compared with the motility for studying any correlation between the motility and fertilizing ability. Semen was processed for separation of spermatozoa into two categories, those demonstrating excellent motility and those having sluggish motility by means of differential centrifugation and repeated washing with BWW medium of the same samples. No correlation between motility and fertilizing ability was observed when the presence of swollen sperm head in the egg cytoplasm was considered as the only criterion of fertilization. However, a positive correlation was obtained when the presence of pronuclei was considered as the criterion for fertilization (Das, R. P. and Warikoo, P. K., unpublished results).

Similar comparison with the sperm count and fertilizing ability of spermatozoa obtained from the semen of monkeys rendered Oligospermic by intra-testicular administration of BCG vaccine demonstrated no significant positive correlation.

Agents producing functional sterility

The results as described earlier indicated the possiblity of interfering with the fertilizing ability of spermatozoa without interruption of spermatogenesis or other sexual functions. Attempts were, therefore, made to induce functional sterility with steroidal and non-steroidal agents so that the effect could be reversible. The main findings are summarized below:

(i) Administration of Depo-Provera in doses of 25 μg/100g body wt. given intra-muscularly every 10 days for 6 months induced sterility in 50% of male rats (Bandopadhyaya *et al.,* 1976).

(ii) Silastic implants containing 17α-hydroxy progesterone caproate induced sterility in 50 % of male rats after 45 days and in 87·5 % after 125 days of insertion of the capsules (Das *et al.,* 1977).

(iii) Comparative studies on the effects of cyproterone acetate (anti-androgen and progestogen), 17α-hydroxy progesterone caproate (a progestogen) and flutamide (pure anti-androgen), indicated that 17α-hydroxy progesterone caproate was the most effective compound in inducing sterility in male rats (Das *et al.,* 1980).

(iv) Treatment with 1·0 mg of STS 557 (17α-cyanomethyl-17β hydroxy estra-4·9 (10)-diene-3-one) daily for 3 weeks had no effect on the weight of the accessory genital organs of male rats. The motility as well as fertilizing ability of spermatozoa were reduced. About 28 % of the seminiferous tubules in testis were partly or completely

devoid of germinal cells. Treatment with 0·5 mg of STS 557 had no significant effect (Warikoo and Das, 1983b).
(v) Administration of *α*-chlorohydrin to male hamsters at a dose of 100 mg/kg body wt. daily for 1 week induced sterility in all (Das and Yanagimachi, 1978).
(vi) Monothioglycerol at a dose of 25 mg/kg body wt., given daily for 5 weeks rendered 4 out of 5 male hamsters non-fertile (Das and Yanagimachi, 1978).
(vii) 5-Thio-D-glucose administered in silastic capsules in male hamsters induced sterility in only 1 out of 6 animals (Das and Yanagimachi, 1978).
(viii) Oral administration of the powder of the seeds of ripe papaya inhibited the fertility in about 40 % of male rats but the weight of the genital organs, the histology of the testis and the pattern of motility of spermatozoa remained unaffected (Das, 1980).

Although no suitable compound was found which could induce reversible inhibition of fertility in all animals, further studies with other steroidal or non-steroidal agents are warranted in this important field. An effective, reversible and safe method may be developed, the effect of which may not necessarily be restricted to post-testicular sites.

Research on primate reproduction

Seasonal variations: Recent researches for the development of contraceptives are being carried out in the primates with the expectation that the results will be replicable in the human. Different species of non-human primates have been used, the most commonly used being rhesus, bonnet and langur. In the rhesus monkey, seasonal variations and summer sterility have been reported in both male and female. Accordingly, it was thought worthwhile to determine the nature of these seasonal variations in terms of some characteristics of the semen profile, and serial studies were carried out in this laboratory on a group of sexually mature male rhesus monkeys maintained under uniform husbandary conditions over a period of two years (Chatterjee *et al.,* 1984)

It was observed that collection of ejaculate was difficult during the non-breeding season. The sperm count per ml semen showed an annual rhythm which varied from 100×10^6 to 400×10^6. The sperm concentration showed a gradual rise from the month of August and reached its peak value in January and the lowest value in June. The abnormal spermatozoa were higher during the non-breeding season as compared to the breeding season. The same was true for the percentage of dead sperm. The motility of sperm was also affected in a similar manner. The hyaluronidase activity of the spermatozoa was reduced during non-breeding season and the level of acrosin resembeled that of hyaluronidase in terms of seasonal variation. The plasma testosterone concentration was maximum during the month of August and much lower during January through July.

The result of the present study has not only provided guidance for properly designing contraceptive studies using rhesus monkeys, but it has also underscored the importance of concurrent collection of similar data on a control group of monkeys throughout the year for proper interpretation of results (Chatterjee *et al.,* 1984).

Induction of reversible sterility by treatment with cyproterone acetate, testosterone enanthate and combination of both steroids: With the object of producing reversible sterility in rhesus monkey mainly through suppression of spermatogenesis, the effects

of intramuscular injections of cyproterone acetate (having anti-androgenic and progestational property) and testosterone enanthate (a long-acting androgen), given separately or in combination, have been studied.

Intramuscular injections of cyproterone acetate 5 mg daily was given to 6 monkeys for 20 weeks. Both the count and motility were reduced significantly. Morphology of spermatozoa was also altered showing an increased proportion of sperms with pin head, coiled tail and amorphous head. Following cessation of treatment, the monkeys became normal after 2–3 months. This treatment affected the synthesis and secretory activity of accessory sex organs.

Seven male monkeys received intramuscular injections of 25 mg/week of testosterone enanthate for 20 weeks. The count decreased significantly from 30 million/ml to 3–6 million/ml of semen. Percentage of abnormal form of spermatozoa increased from 10 % to 80 %. The monkeys became normal after 2 to 3 months of the withdrawal of the drug.

Combined treatment with daily injections of cyproterone acetate plus weekly injections of testosterone enanthate in the above doses was given to 6 monkeys. Spermatozoal count decreased very rapidly and 4 monkeys became azoospermic and the remaining 2 monkeys became very severely Oligospermic. The motility and morphology of the remaining spermatozoa were altered, suggesting infertility. In this group, the volume of semen and coagulum. was significantly increased. Biochemical parameters remained unaltered with initial minor fluctuations. By 5–6 months of cessation of the drug, the monkeys became normal in terms of count, motility and morphology of spermatozoa (Roy *et al.,* 1984; Chatterjee, S., Mukherjee, D. and Roy, S., unpublished results).

Epididymal studies: Rhesus monkeys rendered severely Oligospermic or azoospermic by treatment with cyproterone acetate and testosterone enanthate had spermatozoa in their epididymis but the motility was abnormal. The glycerylphosphorylcholine content in the epididymis and vas tissue was reduced in addition to sperm-bound and luminal plasma sialic acid content. The lysosomal activity as revealed by the acid and alkaline phosphatase in tissue and luminal plasma was increased (Chatterjee, S., Mukherjee, D. and Roy, S., unpublished results).

Electron microscopy: The surface ultrastructure of the spermatozoa of rhesus monkeys treated with cyproterone acetate and testosterone enanthate demonstrated severe undulation and lack of integrity of the acrosomal membrane, occurrence of large pits on the acrosome, lack of clarity of the equatorial segment, swelling of mid-piece and presence of cytoplasmic droplet in the neck region indicating failure of maturation of the spermatozoa (Misro, M. M., Roy, S., Mukherjee, D. and Chatterjee, S., unpublished results).

Reversible vasocclusion

With the object of making male sterilization more acceptable, various means have been tried for the production of reversible vas occlusion. In a systematic study carried out at this centre, the efficacy of occlusion of vas deferens by silastic plugs and intravasal nylon thread have been studied in rats. None of these methods were found satisfactory.

However, from the programme point of view the request for recanalization of vas in

vasectomized individuals was not sufficient to emphasize the need for development of reversible vasocclusion devices or methods. Probably, development of proper referral centres in different parts of the country for microsurgical reanastomosis of vas deferens would be sufficient to fulfil the requirement as and when necessary.

Clinical studies

Male infertility

Semen biochemistry: Investigations on the levels of various biochemical constituents of the semen in relation to the count and motility of spermatozoa are likely to provide useful information on the contribution of different components of the reproductive organs to the seminal pool, valuable guides for proper diagnosis and rational management of their disorders as well as leads for the development of new approaches for contraception. Relative sensitivity of different seminal constituents to androgenic stimulation and deprivation may help in determining the differential sensitivity of the genital organs in the human. Studies were, therefore, carried out in human males to correlate the sperm count and motility with the levels of sialic acid, cholesterol, ascorbic acid, glycerylphosphorylcholine (GPC), fructose, pyruvic acid, lactic acid, and the activity of lactic dehydrogenase, acid phosphatase, alkaline phosphatase and transaminases in the semen under varied clinical and experimental conditions. The results of these studies have been published earlier (Das and Roy, 1976a,b; Das *et al.,* 1975a,b, 1976; Roy and Chatterjee, 1979a,b; Roy *et al.,* 1975, 1976, 1977, 1982).

Differential diagnosis of obstructive and non-obstructive azoospermia: In the management of infertile males, differentiation between obstructive and non-obstructive azoospermia often become problematical. From our long experience with biochemical and morphological studies on human semen in varied clinical conditions, we have been able to develop a reliable method for making such distinction. The levels of GPC and fructose and the acid phosphatase activity in the human semen indicate the functional status of the epididymis, seminal vesicle, and prostate respectively. The presence or absence of mature and/or immature germ cells indicates testicular contribution to seminal plasma.

The patients presenting with azoospermia are initially treated for about 4–6 weeks with antibiotics and antiinflammatory drugs in order to remove any temporary block caused by infection and inflammation in the genito-urinary tract. The cases where sperms do not appear in the semen after such treatment, are subjected to detailed biochemical and morphological studies of the semen. The levels of GPC and fructose and the acid phosphatase activity and the morphology of germ cells in semen are studied. From the observations made in about 200 subjects, it is noted that such a detailed investigation not only helps in the differential diagnosis between obstructive and non-obstructive azoospermia but it also helps in locating the site of obstruction. Furthermore, in cases with non-obstructive azoospermia the nature of the immature germ cells in the semen indicates the nature of spermatogenic arrest, which is further confirmed by histological studies of testicular biopsy (Roy, S. and Banerjee, A. K., unpublished results).

Hormonal therapy

A thorough diagnostic procedure has been developed for determining the cause of infertility in the male. In case of non-obstructive azoospermia, treatment with clomiphene citrate or androgen was tried with very little or no success. In cases of severe oligospermia, rebound therapy using high doses of long-acting tesosterone with or without estrogen were tried with some success. In cases of oligospermia associated with varicocele, glucocorticoid therapy with daily single dose of 0·5 mg dexamethasone given at midnight was tried. Some of these cases responded partially (Roy *et al.,* 1976b). Others were subjected to surgery.

An analysis of 140 cases of oligospermia who received Unitestron depot (l00mg/month) revealed that 28 individuals could impregnate their wives after the treatment (Taneja *et al.,* 1976). Same treatment in three patients with poor sperm motility resulted in restoration of fertility in two of them. Another group of 26 Oligospermic cases were treated with 50 mg of testoviron depot thrice weekly for 4 weeks, followed by 1 mg of stilbestrol daily during the 4th and 5th week. Three of them had restoration of fertility.

Prevalence of infertility

There is no reliable estimate of the prevalence of the infertility in India. The definition of the term also varies. According to the demographic literature, it means 'childlessness at the end of reproductive life span'. According to the WHO definition, it means ‘inability to achieve conception within two years of unprotected sexual cohabitation’. Recently, the Institute has completed a study on the prevalence of infertility in different population groups in the States of Uttar Pradesh, Maharashtra and Himachal Pradesh under the Infertility Task Force of the I.C.M.R., which has been designed and coordinated by this Institute. The study was undertaken with the following objectives: (a) to study the geographical distribution of the problem of involuntary infertility—both primary and secondary; and (b) to identify various socio-cultural, biological and demographic factors associated with involuntary infertility.

The details of the findings are being published separately. The prevalence of primary infertility varied from 3 to 4 % for most of the population groups with an average of 3·7 %, excluding rural Maharashtra where prevalence of infertility was significantly higher (6·1 %). The prevalence rate of secondary infertility was much higher in hilly areas of height more than 4,000 feet (37·6 %) compared to other areas (13–21 %). Rural-urban differential did not exist in secondary infertility. Secondary infertility in rural plains was found to be significantly higher (21 %) than the hills below 4,000 feet height (13·3%).

Establishment of baseline norms of seminal characteristics associated with fertility

The existing procedures for determination of fertility potential of human males are largely emperical. The parameters used for such determination are mainly the count, motility and morphology of the spermatozoa. The sperm count and motility show wide variations among individuals and within individuals. The variations may also be due to different laboratory situations including the procedures followed and experience and

skill of different investigators. The 'normal' range may also be influenced by various other factors, which may be geographical or ethnic, and it may vary with different population groups. While carrying out semenological investigations, it is necessary to know whether the observations fall within or outside normal range. However, the normal range in regard to seminal parameters compatible with fertility potential has not yet been studied in the Indian context, and this needs to be delineated properly.

Male contraception

Duration of residual spermatozoa in human semen after vasectomy

In order to have a rational basis for the guidance of vasectomized individuals, the duration of the presence of sperm in the semen after vasectomy was studied through periodical semen analysis in 450 vasectomized cases, out of 1,200 individuals operated on. Semen analysis was carried out once only in 317 cases, twice in 97 and thrice or more in 36 cases. An analysis of the data revealed that in 197 (44 %) cases the semen became sperm-free within 3 months after the operation, in 105 (23 %) cases within 4–6 months, and in 73 (16%) after 6 months. Out of these 375 cases, total number of ejaculations after the operation could be recorded in 191 cases. In 147 (77 %) cases, the semen became sperm-free by 15 ejaculations, in nine cases by 16–19 ejaculations, and in 35 individuals by 28 or more ejaculations. In about 5 % of cases, a few spermatozoa, mostly non-motile, may be found in the semen even three months after vasectomy or after 15 ejaculations following the operation. However, the chances of having pregnancy with these few sperms are very remote. Spontaneous reunion of the vas deferens leading to failure of the operation was found in 0·7% of cases. It is concluded that contraceptive precautions should be taken for at least 3 months or during 15 ejaculations after vasectomy operation. Wherever possible a semen analysis should be carried out after that period (Poddar and Roy, 1976).

Follow-up study on vasectomized subjects

Over a 5 year period (1964–1969), 1,114 individuals were vasectomized by the NIFP doctor at the Institute and associated clinics. A follow-up study was carried out in 1970, by contacting and interviewing the available cases, on the demographic, socio-economic, general health, side effects and psychosexual aspects. Only 538 cases could be contacted.

Over 87 % of the cases belonged to urban areas and about 13 % to rural areas. More than 75 % of the acceptors had 4 or more children and more than 26 % had 6 or more children. Only 5·5 % of the urban cases and 1·5 % of the rural cases had 2 children. About 93 % of the operated persons clearly stated that they were satisfied with the procedure, and more than 71 % of the individuals recommended this method to others (Roy *et al.,* 1976b; Poddar and Roy, 1976).

Critical review of the state of knowledge on vasectomy, vasocclusion and vasanastomosis

The current status of knowledge in various aspects of vasectomy, vasocclusion and vas reanastomosis has been critically reviewed with the object to identify gaps in the

knowledge and to indicate areas where future research may be profitably encouraged. The areas covered are anatomical and physiological aspects of the vas deferens, vasectomy operation and post-operative care, its complications, failure, psychosexual effects, systemic effects, effects on reproductive system, reversibility of vasectomy operation and development of reversible vasocclusion techniques. This critical review along with major findings and conclusions has been published as a monograph (Roy and Taneja, 1976). A few of the most important conclusions are listed below:

(i) The physiological roles of the vas deferens in the storage, motility, metabolism and transport of the spermatozoa, and their regulation remains to be elucidated.
(ii) The time and number of ejaculations required for the disappearance of sperm from the semen after vasectomy vary a great deal. Therefore, the recommendations regarding the duration of the use of condom following vasectomy need to be based on carefully controlled studies.
(iii) The incidence of the morbidity, reported general satisfaction and alteration in sexual behaviour following vasectomy varies depending upon the interpretation by the patient and the physician. Most of the complications are transient and do not appear to be serious.
(iv) The differences may be due to several factors such as the motivation and preparedness of the acceptors, their socio-economic and cultural background, the incidence and perception of post-operative complications, the rate of infant and maternal mortality, remarriage etc.
(v) A large majority of men have reported general satisfaction and no adverse change in sexual behaviour. Proper education, high motivation, sympathetic environment, and spontaneous agreement by both the partners may ensure better long-term outcome.
(vi) The technique of reanastomosis of vas deferens needs to be improved and standardized. The causes of low pregnancy rate following reanastomosis of vas require further research.

Studies with cyproterone acetate and testosterone enanthate

Effects of cyproterone acetate on reproductive functions inhuman males: Androgens play an important role in the regulation of male reproduction including stimulation of spermatogenesis in the testis, maturation of spermatozoa in the epididymis and maintenance of the function of accessory sex organs. High doses of cyproterone acetate, an anti-androgen with progestational property, suppresses spermatogenesis and libido in animals as well as human beings. Administration of micro doses of cyproterone acetate to laboratory animals had been reported by other investigators to selectively interfere with the epididymal function and thereby produce reversible functional sterility. In view of the above, low doses of cyproterone acetate were administered orally to human volunteers to study whether it would selectively inhibit epididymal function and whether it could be used as a post-testicular anti-fertility drug (Roy *et al.,* 1976a).

The drug was administered to two groups of volunteers in doses of 5 and 10 mg respectively for 28 weeks after a control period of 18 weeks. Semen samples were collected from them fortnightly and analysed for the count, motility and morphology

of the spermatozoa, and for seminal biochemistry. Blood samples were drawn every four weeks for determining the levels of testosterone and for assessing the liver and kidney functions. These studies were carried out before, during and after drug therapy.

During drug therapy in both the groups, there was a significant decrease in the count and motility of the spermatozoa, and an increase in the percentage of immotile or dead as well as immature and abnormal spermatozoa. The lowest sperm count encountered during drug therapy in different individuals varied from 4 to 35 million/ml in the first group and 1·7–41 million/ml in the second group; the earliest time when the lowest count was recorded varied from 10th to 22nd weeks of therapy in the first group and from 6th to 14th weeks in the second group. Those who had initial low counts in the control period responded more rapidly, and decreased counts varied from one to few millions/ml.

Following cessation of drug therapy, the count and motility as well as the percentage of immature and abnormal spermatozoa returned almost to the. control level by the 18th week.

In 4 volunteers from each group, the penetrating ability of the spermatozoa through the pre-ovulatory cervical mucus was assessed at two weeks intervals during 16th to 28th weeks of drug administration and during post-therapy period. In all the cases, the penetrating ability of the spermatozoa through the cervical mucus was markedly decreased during drug therapy, and returned to normal by the 12th week in post-therapy period.

In both the groups, there was a rapid decrease in the acid phosphatase activity and in the levels of GPC and sialic acid in the semen during treatment, indicating an inhibition of the functions of the accessory sex organs. The acid phosphatase activity was most sensitive to the treatment but the level of fructose was not changed. During post-therapy period, the biochemical constituents returned to the control level by the 12th week.

The blood testosterone level was decreased during drug administration in both the groups. Coital frequency and status of libido were not altered. There was no change in the liver and kidney function tests.

It is noted that cyproterone acetate caused a partial suppression of spermatogenesis, inhibited the motility of the spermatozoa and compromized with the cervical mucus penetrating ability of the remaining motile spermatozoa in the ejaculate. The actions of the drug were manifested simultaneously at multiple sites and contrary to the claim made in rats by other investigators, no selective inhibition of epididymal function was demonstrable in the human. The results indicated that low doses of this drug might be used as a reversible contraceptive for the human males. The original findings of this study, which were supported by the WHO concurrently in two centres in the world, raised hope that an important break-through in this difficult field of endeavour would be possible (Roy *et al.,* 1976a,; Roy and Prasad, 1976; Roy and Chatterjee, 1979a).

Further elucidation of the site and mode of action of cyproterone acetate in human males: In the preceding study, it was postulated that the main action of the low dose of cyproterone acetate was probably mediated through androgen deprivation at the level of the target tissues. Androgen deprivation was produced in two ways—by decreasing the secretion of androgen from the testis and by competitive inhibition of androgen

action at the receptor sites in the target tissues. To produce further evidence to this hypothesis the following experiment was undertaken (Roy and Chatterjee, 1979b).

After completing the investigations in the control period, 10 mg of cyproterone acetate (CPA) was administered daily to three normal human volunteers. Following 12 to 18 weeks of drug therapy in all of them, the count and motility of the spermatozoa were significantly decreased and the immature and abnormal forms were increased; the levels of acid phosphatase, and GPC in the semen were decreased. At this stage daily oral administration of an androgen (75 mg mesterolone) was started in addition to CPA therapy. After a short period, the count and motility of the spermatozoa started increasing and the immature form decreased; the level of seminal acid phosphatase was increased, but not the GPC.

The results indicated that androgen deprivation caused by CPA inhibited spermatogenesis in the seminiferous tubules, sperm maturation in the epididymis, and the secretory activity of the prostate and epididymis. Concurrent administration of androgen counteracted the inhibitory effects of cyproterone acetate, probably by competing at the receptor sites in the testis, epididymis and prostate. Incidentally, these findings also provided an evidence for a direct stimulatory effect of androgen on the germinal cells.

Reversible contraception in human male by combined administration of cyproterone acetate and testosterone enanthate: From the evidence available in the studies carried out by Roy *et al.* (1976a; 1979a,b) and Hamerstein *et al.* (1976, 1977), two possible modes of action of CPA were envisaged: (i) suppression of pituitary gonadotropin secretion due to its strong progestational property, and (ii) androgen deprivation at the target sites resulting from its capacity to successfully compete for binding with androgen receptors, in addition to lowering of blood level of androgen. Because of the inhibitory action of CPA at multiple sites it was thought that low doses of CPA might be used as a reversible contraceptive in the male (Roy *et al.,* 1976a; Roy and Chatterjee, 1979a). However, one of the main objections of such use was the possible side effects of long-term androgen deprivation in population groups with marginal nutritional status in developing countries.

In order to obviate the side effects of androgen deprivation and with a view to achieving possible additive effects as in the case of other reported progestin-androgen combinations, it was thought worthwhile to study the effects of combined administration of CPA and testosterone enanthate (TE), a long-acting androgen, having a potent gonadotropin-suppressing property (Roy *et al.,* 1982, 1984). Three groups of human volunteers were studied under three different types of treatment regimen, which followed a control period of 8–10 weeks. One group received 20 mg CPA daily orally, the second group received 250 mg of TE intramuscularly once fortnightly, and the third group received the combined treatment with 20 mg CPA daily and 250 mg TE fortnightly over a period of 20 weeks. The volunteers were followed up for 20 weeks during the post-therapy period.

From the analysis of the data, it is revealed that combined therapy with CPA and TE had additive effects on the count and motility of the spermatozoa. Five out of six volunteers becamse azoospermic by 6–8th weeks of treatment. One volunteer who did not become azoospermic, had only 0·01 million sperm count/ml of semen and these

spermatozoa showed no or sluggish motility and abnormal morphology. On the other hand, treatment with CPA or TE alone failed to induce azoospermia in more than one out of four subjects. Furthermore, in the combined treated group, azoospermia was induced much earlier and persisted throughout the treatment period. In this group, the activities of the acrosomal enzymes, *viz.,* hyaluronidase, acrosin and acid phosphatase, were significantly decreased. Although there was a significant decrease in the level of luteinizing hormone in the peripheral blood, the testosterone level remained largely within normal range. No abnormal changes were observed in the activities of SGOT and SGPT, and in the levels of urea, creatinine and cholesterol in the blood. There was no alteration in the libido and coital frequency. The secretory activity of the accessory sex glands as revealed by the levels of GPC, fructose and sialic acid and the acid phosphatase activity in the semen, remained within normal range in the combination treatment group, whereas with CPA treatment a reduction in secretory activity was recorded. The volunteers receiving TE treatment alone showed an increase in the levels of fructose and GPC and in acid phosphatase activity (Roy *et al.,* 1982, 1984).

The results of this study have demonstrated that combined administration of cyproterone acetate and testosterone enanthate may be an effective reversible hormonal contraceptive for the male. It may be pertinent to mention that over the past 16 years or so a large variety of hormonal regimens have been tried by different groups of investigators throughout the world. Such studies have been carried out under the auspices of the WHO, International Committee for Contraceptive Research, the Population Council, the National Institute of Health, USA and others. None of these regimens have been found effective.

The present approach developed at the NIHFW may prove to be an important breakthrough for reversible male contraception. In fact, in a recent meeting of the Joint Steering Committee of the WHO and Indian Council of Medical Researrh for Male Fertility Regulation held in Bombay, it has been recommended that this method may be put on extended clinical trial with the ultimate hope for field trial at a later period.

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