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EXPERIMENTAL AND CLINICAL EVALUATION OF CONTRACEPTIVE AGENTS

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ABSTRACT: Contraceptive agents are drugs or devices used by women and men in the reproductive age group, to prevent unintended pregnancies. Although various contraceptive agents are available for women and men, they are associated with health risks or lack of efficacy, and thus are insufficient to cater to the needs of the diverse target population with varying contraceptive requirements according to their socio-cultural background and individual preferences. Thus, there is a need to develop newer contraceptive agents with better risk benefit profile and greater acceptability. Thorough understanding of the complex reproductive physiology would help us in identifying various targets for developing efficacious and safe contraceptive agents. Agents with contraceptive potential need to be evaluated for its efficacy and safety in various preclinical and clinical studies before it could be granted marketing authorization. This review discusses in brief about the various *in vitro* tests and *in vivo* methods (animal models) used for evaluation of the contraceptive agents. Contraceptive agents for women can be screened for their estrogenic, anti-estrogenic, progestational, anti-progestational, anti-ovulatory and anti-implantation activity in various animal models. Contraceptive agents for men can be screened for their ability to inhibit spermatogenesis and androgenic or anti-androgenic activity by different animal models. This review also discusses about the clinical evaluation of the contraceptive agents in various phases of clinical trials with emphasis on the measurement of efficacy, safety and acceptability of the contraceptive agents.

INTRODUCTION: Contraceptive agents are drugs or devices used by women and men in the reproductive age group, to prevent unintended pregnancies. Every couple has the right to choose the timing and size of their family. Contraceptives are intended for use in healthy and fertile couples who are diversified based on their social, cultural and economic background.

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Hence, there is a need for a wide range of contraceptive methods which suit the individual needs of the diversified target population and thus ensure provision of the best method for each individual based on their medical conditions and individual preferences. Thus, there is an unmet need to develop new safe, effective and acceptable contraceptive methods. The development of more number of contraceptive agents will have a positive impact on human health and well being.

Currently available contraceptives for females can be classified into hormonal methods and non-hormonal methods. The hormonal methods for women include contraceptive pills, injectable preparations, implants, hormone-releasing devices

(intrauterine contraceptive devices and vaginal rings) and post-coital pills. The non-hormonal methods in women include sterilization, intrauterine contraceptive devices, barrier methods (diaphragm and female condom), spermicides and natural methods (calendar and lactational amenorrhoea) [1]. The contraceptive methods for males include vasectomy and barrier methods like condom [1]. These contraceptive methods have risk of high failure rate, health risks or inconveniences associated with its use. Also, it is desirable to develop contraceptives with additional non-contraceptive benefits like regularization of menstrual cycle in women, prevention of sexually transmitted diseases *etc*. A good understanding of the reproductive physiology in males and females would help in identifying newer drug targets for development of safe and efficacious contraceptive agents.

Physiology of Reproduction:

Physiology of Reproduction in Females: Unlike the male reproductive system, the female reproductive system undergoes cyclical changes in order to prepare for implantation of the fertilized ovum.

Menstrual Cycle: Menstrual cycle occurs in primates including humans. The menstrual cycle has two phases, namely the follicular phase or proliferative phase and secretory phase or luteal phase. In the follicular phase, the primordial follicle in the ovary develops to form graffian follicle which produces estrogen. During this phase, the endometrium proliferates and cervical secretions are thin and stringy. Estrogen produced from the ovarian follicles produces a positive feedback to the pituitary gland and thus causes LH surge which leads to ovulation. This marks the onset of luteal phase where corpus luteum is formed and it secretes progesterone. Under the influence of progesterone, the endometrial glands become more tortuous and it becomes conducive for implantation of fertilized ovum. If ovum is not fertilized, corpus luteum involutes and the levels of estrogen and progesterone fall, thus leading to menstruation. Menstruation is a process of desquamation of the superficial layers of the endometrium. The normal duration of menstrual cycle is 25 - 30 days [2]. The endometrium is shed in the menstrual cycle, whereas in estrus cycle it gets reabsorbed.

Estrus cycle: [3] It occurs in non-primates such as dogs, cats and rodents. Rats are preferred in reproductive studies due to their short estrous cycle, which is completed in 4-5 days. The estrous cycle is characterized by morphological changes in ovaries, uterus and vagina. It has the following four phases:

Proestrus Phase: This is the first stage of estrus cycle. It is similar to the follicular phase of menstrual cycle in humans. Follicles develop in the ovaries under the influence of gonadotropins and synthesize estrogens. Thus, the level of estrogen increases in this phase. The vaginal smear is characterized by nucleated epithelial cells. This phase lasts for about 12 hours.

Estrus Phase: In this phase, there is maximum estrogen secretion and the uterus is filled with sanguinous fluid. This is the period of sexual receptivity and ovulation occurs spontaneously. The vaginal smear contains squamous cornified epithelial cells.

Metestrus Phase: This is the postovulatory phase. Ovary contains corpus lutea which secretes progesterone. In this phase the vaginal smear contains cornified epithelial cells and leucocytes. This phase lasts for about 21 hours.

Diestrus Phase: In this phase, the corpus lutea regresses and there is fall in the levels of estrogen and progesterone. This is the longest phase of the cycle which lasts for 57 hours. The vaginal smear contains only leucocytes.

Physiology of Reproduction in Males: The hypothalamo-pituitary axis controls the male reproductive functions. The testis contains sertoli cells and leydig cells. The sertoli cells are involved in spermatogenesis and are stimulated by the FSH produced from the anterior pituitary. Similarly, LH produced from the anterior pituitary gland acts on LH receptors present on the leydig cells and promotes testosterone synthesis [2].

Experimental Evaluation of Contraceptive Agents for Females: Anti-fertility action of drugs

in females could result from inhibition of ovulation, prevention of fertilization, interference with the transport and implantation of fertilized ovum or destruction of early implanted embryo. Estrogen and progesterone are involved in co-ordination of events involved in ovulation, ovum transport and implantation. Thus, hormonal imbalance caused due to administration of estrogenic, progestational, anti-estrogenic and anti-progestational compounds could produce anti-fertility activity [4].

***In-vitro* Methods:** [5]

Estrogen Receptor Binding Assay: Estrogenic activity of the test compounds are determined by its ability to bind with estrogen receptors. Mouse uteri or human endometrium serve as sources of estrogen receptors and estradiol is used as the standard.

Gestagen Receptor Binding Assay: Progesterone activity of the test compounds are determined by its ability to bind with progesterone receptors. Progesterone receptors can be obtained from uteri of estrogen primed rabbits and human uteri obtained after hysterectomy. Progesterone is used as the standard.

***In-vivo* Methods:**

Evaluation of Estrogenic Activity:

Uterine Weight Assay: [6] This test is based on the principle that estrogenic compounds increase protein synthesis and thus increase the uterine weight. Ovariectomized female albino rats were given the test drug or standard (estradiol) intramuscularly for 3 days. On the 4th day, the animals were sacrificed and the uterus was dissected out from the abdomen. The uterine contents were cleared and the uterine weight was measured immediately in wet state. The uterus was placed in oven at 100 °C and dehydrated. It was again weighed to calculate the increase in dry weight. An increase in uterine weight is an indication of the estrogenic property of the test drug.

Vaginal Cornification Assay/Allen-Doisy Test: [4, 7] Ovariectomized female albino rats were used for the study. The test drug is given orally twice daily at various doses for 3 days. Estradiol is taken as the standard. On 4th day, vaginal smears were taken with a saline soaked cotton swab, and smeared over a glass slide. It was stained with 5% aqueous methylene blue solution for 10 minutes and examined under a microscope. The compounds having estrogenic activity shift the animals into estrous phase, skipping the other stages. If vaginal smears of rats show non-nucleated cornified epithelial cells, characteristic of estrus phase, it confirms the estrogenic nature of the test drug.

Chick Oviduct Method: [5] This method is used for screening of estrogenic compounds. The estrogenic compounds increase the weight of chick oviduct in a dose dependent manner. Pullet chicks are injected subcutaneously with standard (estradiol) or the test drug twice daily at varying doses for 6 days. Animals are sacrificed 24 hours after the last injection and the weight of the body and oviduct are measured. Increase in weight of oviduct in the test group suggests that it has estrogenic potential.

Evaluation of Anti-Estrogenic Activity: The same assay techniques used for evaluation of estrogenic compounds are employed. The compounds with anti-estrogenic property will antagonize the physiological effects of estrogen like increase in uterine weight and vaginal cornification.

Evaluation of Progestational Activity:

Pregnancy Maintenance Test in Rats: [5] This test is based on the principle that progesterone is required for maintenance of pregnancy. Ovariectomy performed in the first half of pregnancy leads to termination of pregnancy due to progesterone deficiency. However, ovariectomy done in the second half of pregnancy does not lead to abortion since the placenta produces progesterone needed for sustaining the pregnancy. Ovariectomy is done on day 8 of pregnancy in female Sprague-Dawley rats. The test drug is given subcutaneously once daily for 13 days, starting from the day of ovariectomy. On day 21 of gestation, the animals are sacrificed and the number of implantation sites and number of live embryos are noted. Normal pregnant rats contain around 11 implantation sites and 10 live embryos.

Deciduoma Formation in Rats: [4] Traumatized estrogen-primed endometrium of rats responds to progestational agents by formation of maternal/ placental tumour at the site of uterine injury.

Female albino rats are ovariectomized and primed with estradiol for 4 days and the test drug is injected once daily for 9 days. On the 5th day of test drug administration, 1mg of histamine dihydrochloride is injected into the lumen of one uterine horn of the rat. After a period of 24 hours after last dose of drug, the animals are sacrificed; uterine horns are dissected, weighed and histologically examined for the presence of deciduoma.

Clauberg and McPhail Test in Rabbits: [5] This is based on the principle that progesterone causes proliferation of the estrogen primed endometrium in rabbits. Sexually immature female rabbits are primed with estradiol for 6 days and on day 7, the test drug is administered once daily for 5 days. After a period of 24 hours after the last dose of drug administration, the animals are sacrificed and their uteri are dissected. Histological examination of the middle portion of uterine horn is done for assessing the degree of endometrial proliferation by McPhail score.

McPhail Score:

0- Ramification of the uterine mucosa but no proliferation.
1- Slight proliferation of the uterine mucosa.
2- Medium proliferation with slight additional ramification.
3- Pronounced proliferation of the uterine mucosa.
4- Very pronounced proliferation of the uterine mucosa and pronounced ramification.

Endometrial Carbonic Anhydrase Assay in Rabbits: [5] It was found that the amount of carbonic anhydrase in uterine endometrium of rabbits is dependent on the amount of progesterone produced from the corpus luteum. Immature female albino rabbits are primed with estradiol and the test drug or standard is given as in Clauberg test. After 24 hours of last dose of the drug, the animals are sacrificed and uteri are dissected. The carbonic anhydrase activity of the endometrial extract is determined. Exogenous progestinal agents increase the carbonic anhydrase activity of the endometrium.

Prevention of Abortion in Oxytocin Treated Pregnant Rabbits: [4] This method is based on the fact that progesterone prevents abortion induced by oxytocin in pregnant rabbits. On 30th day of pregnancy in rabbits, oxytocin at a dose of 10U is injected intravenously. Twenty four hours before administration of oxytocin, the test drug is injected. The control animals will abort within 2 to 30 minutes of oxytocin administration. If the test drug has progestational action, it will prevent oxytocin induced abortion in rabbits.

Evaluation of Anti-Progestational Activity: The same assay techniques used for evaluation of progestational compounds are employed. The compounds with anti-progestational property will antagonize the physiological effects of progesterone like deciduoma formation in rats and increased proliferation of estrogen-primed endometrium of rabbits.

McGinty Test (Evaluation of Anti-progestational Activity in Rabbits):[6] Sexually immature female rabbits are primed with subcutaneous estradiol for 6 days. On the 7th day, the rabbits are anaesthetized with ketamine and abdomen is opened and uterus is dissected. The test drug and progesterone are injected directly into the uterine segment and the muscular layer and abdominal incision are sutured. The animals are allowed to recover and 3 days after the procedure, the animals were sacrificed. The uterus of the animals were dissected and examined histologically for McPhail score. Inhibition of progesterone induced proliferation of estrogen-primed endometrium indicates anti-progestational activity.

Evaluation of Anti-Ovulatory Activity:

HCG Induced Ovulation in Rats: [6] This test is based on the principle that immature female albino rats do not exhibit spontaneous ovulation and administration of human chorionic gonadotropin (HCG) induces ovulation in 2 days. Immature female albino rats are given various doses of the test drug, following which HCG is administered. The animals were sacrificed after 2 days of HCG administration; their ovaries are dissected and examined histologically. Compounds with anti-ovulatory activity inhibit the HCG induced ovulation.

Cupric Acetate Induced Ovulation in Rabbits: [6] Sexually mature rabbits ovulate within a few hours

after intravenous administration of chemicals like cupric acetate. Mature rabbits are housed singly (isolated) for a period of 21 days to make sure that the rabbits are not pregnant. The test drug is administered and 24 hours later, cupric acetate is given intravenously. After a period of 24 hours after cupric acetate administration, the animals are sacrificed and ovaries are dissected. Both the ovaries are histo-pathologically examined for the total number of ovulation points. Drugs with antiovulatory action have lesser number of ovulation points compared to that in the animals of the control group.

Evaluation of Anti-implantation activity: [6, 8] Fertile female albino rats in estrous stage are made to mate with fertile male rats in ratio of 3:1. The vaginal smear from each female rat is examined for the presence of spermatozoa from the next day onwards. Once mating is confirmed, it is considered as the first day of pregnancy and the female rats are transferred to separate cages, housed singly and monitored. The test drug is given orally to the female rats once daily at various concentrations during the gestational period. On the 10th day of gestation, laparotomy is performed under anaesthesia and the number of corpora lutea (CL) in both the ovaries and the number of embryos implanted in both the uterine horns is noted. The organs are replaced back and abdominal incision is sutured in layers. The animals are put back in their cages and pregnancy is allowed to progress to complete the gestation. The number of litters delivered (if any) is counted. The following formula could be used to calculate the pre and post implantation losses and anti-fertility activity of the test drug.

Pre-implantation loss = No. of CL on day 10 – No. of implants on day 10
Post-implantation loss = No. of implants on day 10 – No. of litters delivered

$$\% \text{ Pre-implantation loss} = \frac{\text{No. of CL} - \text{No. of implants}}{\text{No. of CL}} \times 100$$

$$\% \text{ Post-implantation loss} = \frac{\text{No. of implants} - \text{No. of litters}}{\text{No. of implants}} \times 100$$

$$\% \text{ Anti-fertility loss} = \frac{\text{No. of CL} - \text{No. of litters}}{\text{No. of CL}} \times 100$$

Experimental Evaluation of Contraceptive Agents for Males: Anti-fertility drugs may cause sterility in male laboratory animals by inducing aspermia, oligospermia or formation of non-functional spermatozoa. Also, hormonal imbalance caused by administration of androgens and anti-androgens may produce anti-fertility activity.

***In-vitro* Methods:** [5] Androgen receptor binding assay - Androgenic activity of the test compounds are determined by its ability to bind with androgen receptors. Mouse kidney or rat ventral prostate serve as sources of androgen receptors and testosterone is used as the standard. Inhibition of 5 α reductase – Compunds inhibiting the activity of 5α- reductase have anti-androgenic action since 5α-reductase is involved in the conversion of testosterone to dihydro-testosterone. Rat prostate or human prostate from benign prostatic hyperplasia patients serve as source of 5α- reductase enzyme.

***In-vivo* Methods:**

Agents Interfering with Spermatogenesis:

Fertility Test: [4] This test is based on the assumption that contraceptive agents reduce the average litter size in rats. Groups of 5 to 10 male rats with proven fertility are administered the test drug. Then each male rat is placed along with fertile female rats in the ratio of 1:3. Daily vaginal smears of the rats are examined for presence of spermatozoa. By the end of each week, all females would have passed through one estrous cycle (sexually receptive) and copulation should have occurred in most pairings. If insemination is not detected, it could be due to failure to mate (loss of libido) or aspermic copulation. In case of failure to mate, the normal estrus cycle will continue in the female rats. In case of aspermic copulation, pseudo-pregnancy results and it can be identified by the presence of leucocytic vaginal smear for 10 to 14 days.

The average litter size is calculated using the following formula:

$$\text{Average litter size} = \frac{\text{Total number of litters}}{\text{Number of females mated}}$$

If the test drug has anti-fertility effect, it reduces the average litter size in rats.

Cohabitation Test: [4, 9] This is an alternative method of testing fertility using lesser number of female rats compared to fertility test. This test is based on the principle that drugs with contraceptive potential increase the time interval required for litter production when a fertile male rat treated with the test drug is cohabited with 2 fertile female rats. Fertile male rats are administered the test drug and are kept for mating with female rats in the ratio of 1:2 till both the female rats deliver the litters. The date of mating is calculated from the date of parturition (gestational period in rats is approximately 22 days). Anti-fertility drugs prolong the time interval required for litter production.

Androgenic Activity:

Capon's Comb Method: [7] This method is based on the principle that androgens increase the growth of capon's comb. The capons were castrated to stop the endogenous androgen production and thus inhibit the growth of their comb. The length and height of the capon's comb were measured in millimeters. The test drug or standard (testosterone) is injected intramuscularly into the pectoral muscle of the capon once daily for 5 days. After a period of 24 hours after the last dose of the drug, the length and height of the capon's comb are measured again. Increase in length and height of the capon's comb is an indication of androgenic activity of the test compound.

Weight of Androgen Dependant Organs in Rats: [5] This test is based on the principle that androgens increase the growth of ventral prostate, seminal vesicle and levator ani muscle in rats. Orchiectomized immature male rats are administered the test compound or standard (testosterone) orally or subcutaneously once daily for 10 days. On the 11th day, the animals are sacrificed and ventral prostate, seminal vesicle and levator ani muscle are dissected and weighed. The ratio of organ weight to body weight is calculated for each animal and each organ and dose response curves are plotted. The increase in the weight of ventral prostate and seminal vesicle is an index of androgenic activity and the increase in weight of levator ani muscle is an index of anabolic activity of the test drug.

Anti-Androgenic Activity: The same assay techniques used for evaluation of androgenic compounds are used to demonstrate anti-androgenic activity. The compounds with anti-androgenic property will antagonize androgen induced increase in the growth of capon's comb and increase in the growth of ventral prostate, seminal vesicle and levator ani muscle in rats.

TABLE 1: ANIMAL MODELS FOR CONTRACEPTIVE AGENTS IN MALES

Animal models for contraceptive agents in females	Animal models for contraceptive agents in females
Uterine weight assay	Fertility test
Vaginal cornification assay	Cohabitation test
Chick oviduct method	Capon's comb method
Pregnancy maintenance test in rats	Weight of androgen dependant organs in rats
Deciduoma formation in rats	
Clauberg and McPhail test in rabbits	
Endometrial carbonic anhydrase assay in rabbits	
3. Prevention of abortion in oxytocin treated pregnant rabbits	
McGinty test	
HCG induced ovulation in rats	
Cupric acetate induced ovulation in rabbits	
Anti-implantation activity evaluation	

Clinical Evaluation of Contraceptive Agents: Once a contraceptive agent is found to be efficacious and non-toxic in preclinical studies, it has to be evaluated in humans to confirm its safety and efficacy before it can be approved for clinical use in the target population. A clinical trial with the new contraceptive agent begins with the approval of the IND (Investigational New Drug) application submitted to the FDA (Food and Drug Administration). Human trials with the contraceptive agent begin after a 30 days waiting period, if the FDA does not reject the application[10].

How Contraceptives Differ from Other Investigational Agents? [10] Contraceptives are to be used by healthy people

- Target populations are large and diverse.
- Contraceptives are going to be used for prolonged periods (*e.g.* approximately 30 years of the reproductive life for a women).
- Contraceptives need to have very high safety profile and good efficacy to have a favourable risk-benefit balance. However, no contraceptive agent is 100% effective and risk of pregnancy is still present.

Phase I Trials: Phase I studies are conducted in healthy volunteers to study the safety and tolerability of the drug. Phase I studies are also used to assess the pharmacokinetic properties of the drug. It is conducted in 20 to 80 healthy volunteers, around 10-20 volunteers for each dose level in a single centre in a closely monitored set up [11]. The participants of the study for testing contraceptives for women include healthy, fertile, sexually active participants of the reproductive age group (19 to 39 years) with regular menstrual cycles (25 to 30 day length with less than 7 days of menstrual flow and absence of intermenstrual bleeding), who are IUCD users or sterilized, and therefore not at risk of becoming pregnant. Pregnant and lactating women, participants with cardiovascular, hepatic, renal, endocrine or gynecologic diseases are excluded from the study [12]. For evaluation of contraceptive agents agent for which reversibility of fertility is not assured, it is advisable to include women >25 years of age and who have children [1]. The phase I trials help to establish the safety of the contraceptive agent and helps to calculate its pharmacokinetic parameters.

Phase II Trials: Phase II trials are conducted in the target population to assess the efficacy and safety of the contraceptive agent. Phase II studies are conducted in 100 to 200 participants. The goal of phase II studies is to establish the mechanism of action and identification of optimum dosage regimen for the contraceptive agent. The acceptability of the contraceptive agents to the target population is also evaluated in phase II trials. Inclusion criteria for women in phase II trials are different from that of phase I trials.

The Inclusion Criteria for Phase II Trials Include: [13]

- Healthy, fertile, sexually active participants of the reproductive age group (19 to 39 years) who do not want to get pregnant (want to limit their family size) but understand that there is a risk of conception.
- Should have had three consecutive, normal menstrual cycles immediately prior to enrolment.
- Should not have used steroidal contraceptives in past 3 months.
- Should not have used IUCD in the past 1 month

Inclusion Criteria for Men: Males in same age range, with no evidence of any disease and have two normal semen analysis [12]. Knowledge of the mechanism of action of the contraceptive agent helps in choosing the endpoints in assessment of pharmacodynamic effects. The direct assessment of efficacy in small trials is not possible since pregnancy is a rare event with highly efficacious contraceptive agents. Hence in phase II trials, surrogate end points are commonly employed to assess the efficacy of a contraceptive agent for prevention of pregnancy.

Phase III Trials: Phase III trials are confirmatory trials which are used to confirm the efficacy, safety and acceptability of the drug in comparison to the current standard contraceptive(s) in the target population [1]. Phase III trials are usually randomized controlled double blinded study with active control. However, clinical trials comparing implants, IUCD and other devices cannot be blinded [1].

Recruitment of Participants: Participants are recruited from the family planning clinics. The couples requesting the contraceptive method under study are screened for eligibility after obtaining informed consent. Principal investigators are specialist in obstetrics and gynecology. Women are requested to come to the clinics at specific time points during the follow up period for assessment of parameters of safety and efficacy. Efficacy of the contraceptive is measured by Pearl index [14].

An open-label multi-centricphase III clinical trial was conducted by Roumen *et al.,* [15] to study the efficacy, tolerability and acceptability of a novel contraceptive vaginal ring releasing etonogestrel 120 µg and ethinyl estradiol 15 µg daily for 3 weeks (followed by 1 week ring-free period) in 1200 healthy women in reproductive age group for 13 cycles. Contraceptive efficacy was assessed by

periodic pregnancy tests. Vaginal bleeding was recorded in menstrual diaries. Participants also documented the number of hours of ring use per day in a separate diary to assess compliance. Acceptability was analyzed using the discontinuation rates and the reasons for discontinuation. The study concluded that the hormone releasing vaginal ring was efficacious with pearl index of 0.6 per 100 women years of exposure. It was well-accepted and well-tolerated with headache, leucorrhoea and vaginitis being the common adverse effects.

Phase IV Trials: Phase IV trials are conducted after the drug has been approved for marketing. Phase II and phase III studies have insufficient number of participants and inadequate study duration to identify rare adverse events like development of cancer, cardiovascular disease or thromboembolic disease, especially with the use of hormonal contraceptive agents. Hence, to identify these rare adverse events and those that occur with chronic use, there is a need for post marketing surveillance and post marketing clinical trials. It can be done by spontaneous adverse drug reaction reports, and epidemiological studies (cross-sectional, case-control and cohort studies) [16].

For example case reports of venous thromboembolism, myocardial infarction and stroke in women taking combined oral contraceptives was the starting point in identification of these adverse effects of combined oral contraceptives. Similarly, case control studies were employed in confirming and refuting the adverse effects of combined oral contraceptives. It also aids in identifying the subgroup of users of combined oral contraceptives like smokers, who had higher risk of developing myocardial infarction [11].

Evaluation of Contraceptive Effectiveness: The accurate measure of contraceptive failure is the occurrence of pregnancy (serological and clinical diagnosis of pregnancy) during period of time in which the woman is using a contraceptive agent. The occurrence of pregnancy could be due to failure of contraceptive method or due to lack of use or incorrect use of the contraceptive method. The pregnancies resulting due to incorrect use or lack of use of the contraceptive agent has been denoted as "user failures". The pregnancies resulting even after the correct use of the contraceptive agent are denoted as "method failures" [1].

Pearl Index: Pearl index is the commonly used method for reporting the effectiveness of a contraceptive agent. Pearl index represents the number of unintended pregnancies (contraceptive failures) per 100 women years of a contraceptive method's use. It is calculated by the following formula: [14]

$$\text{Pearl index} = \frac{\text{Number of pregnancies X 1200}}{\text{Number of women X Number of months of contraceptive exposure}}$$

Lower the pearl index, higher is the contraceptive efficacy. Pearl index is of two types namely, "perfect use pearl index" and "actual use pearl index". "Actual use pearl index" includes all pregnancies occurring in the clinical trial and all months or cycles of contraceptive exposure. On the other hand, "perfect use pearl index" includes only those pregnancies which occurred with the correct and consistent use of the contraceptive agent and includes only those months or cycles in which the contraceptive agent was used correctly and consistently. The calculated "perfect use pearl index" and "actual use pearl index" are different with contraceptive agents which exhibit high user dependency. For example oral contraceptive pills must be taken daily without missing any dose, to be effective and thus have high user dependency. Whereas, intrauterine contraceptive devices, once inserted will be effective for a period of 10 years. It does not require any action to be performed or remembered by the user during its 10 year efficacy period for prevention of conception. Thus, it is said to be a non-user dependant method, and the calculated perfect use and actual use pearl index are nearly similar [14]. For pearl index calculations, either 12 cycles (for non-cyclic contraceptive methods) or 13 cycles (for cyclic contraceptive methods) constitute one woman year, depending on the duration of one menstrual cycle taken as 30 days or 28 days respectively [16].

Surrogate Marker: The surrogate markers employed depend on the contraceptive method used in the study. The surrogate markers used in contraceptive clinical trials in women are: [13, 16]

- Effect on ovarian function by ovarian ultrasonography (at least two cycles should be studied in each women). Also time to return of ovarian function after discontinuation of the contraceptive method should be studied. The patients have to be followed up for period of 1 year following discontinuation of the contraceptive method.
- Endocrine profile like serum estrogen, progesterone, testosterone and gonadotropin levels.
- Effects on cervical mucous - sperm cervical mucous penetration test.
- Effects on endometrium.

In case of contraceptive clinical trials in men, the drug/ intervention is provided to men, whereas the outcome (pregnancy rate) is to be observed in women, which becomes cumbersome. Therefore, surrogate markers are used in contraceptive clinical trials in men like sperm count, morphology and motility [17].

Evaluation of Safety and Acceptability of Contraceptives: Hormonal contraceptive agents are frequently associated with disturbances in vaginal bleeding pattern. Women participating in the clinical trials should be asked to maintain menstrual diaries. The following information should be noted by the participants in the menstrual diaries: [1]

- Number of episodes of spotting per menstrual cycle (spotting refers to the use of $\leq$ 1 pad/tampon /day).
- Number of bleeding/spotting days per menstrual cycle (bleeding refers to use of $\geq$ 2 pads/tampons /day).
- Amount of bleeding per episode.

Follow up of all pregnancies occurring due to contraceptive failure must be done until the delivery of the baby to study the effect of these drugs on the fetus.

Other parameters to be monitored to assess the safety of the contraceptive agent are:

- Physical examination – Weight, blood pressure, breast and pelvic examination.
- Effect on coagulation, fibrinolysis and platelet function.
- Effects on carbohydrate and lipid metabolism.
- Cervical cytology at baseline and then annually to screen for malignancies.
- Complete blood count, liver and renal function test.
- Effects on bone mineral density.
- Subsequent progeny should be examined for the presence of congenital defects (if patient becomes pregnant).
- Participants should be followed up after the trial to see for resumption of fertility.

In the clinical trials to evaluate anti-fertility agents, it is also essential to measure the acceptability of the contraceptive method. The total number of participants withdrawing from the study (stopping the use of the contraceptive method) is an indication of unacceptability of that contraceptive method. Acceptability of the contraceptive can be assessed by questions to the users regarding satisfaction, willingness to recommend the method to others and to pay to have access to the method [1].

Duration of Contraceptive Trials: The duration of studies to assess the efficacy of the contraceptive agent should be 6 months or more. To judge the efficacy and safety of contraceptive agents, clinical trials involving 1000 women for 6 months with a total of 6000 women months of contraceptive exposure are required [12].

CONCLUSION: Contraceptive agents are used by fertile couples to prevent unintended pregnancies. Although various contraceptive agents are available for women and some for men, they are associated with side effects and thus there is a need for more contraceptives with different mechanisms of action. The new agents with contraceptive potential need to be evaluated for their safety and efficacy in various animal models before they could be tested in humans. Contraceptive agents for women can be screened for their estrogenic, anti-estrogenic, progestational, anti-progestational, anti-ovulatory and anti-implantation activity in various animal models. Contraceptive agents for men can be screened for their ability to inhibit spermatogenesis and androgenic or anti-androgenic activity by different animal models. Evaluation of efficacy and safety of contraceptive agents in humans is done in

various phases of clinical trials. Efficacy of a contraceptive agent is evaluated in terms of pregnancy rate (pearl index) or with the help of surrogate markers like effect on ovarian function, endometrium and cervical mucus in women, and sperm count and motility in men. Safety of the contraceptive agent can be evaluated by monitoring the menstrual bleeding pattern, assessing its effect on coagulation, fibrinolysis, platelet function, liver function test, renal function test and, carbohydrate and lipid metabolism.

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