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REVIEW ON "ASSESSMENT OF ANTIFERTILITY ANIMAL MODELS"

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ABSTRACT

The aim of this review paper is to provide comprehensive summary of in vivo and in vitro Antifertility Models and Antifertility agents. Overpopulation is becoming one of the major global problems causing much influence on the economic, social and natural resources and this intensifies the need of effective birth control measures. This paper also reviews Antifertility activity along with female hormones estrogen and progesterone, which plays important role in hormonal balance in females. In this review paper we have to discusses the various in vivo methods such as sperm motility and count, Vaginal opening, vaginal cornification assay, chick oviduct method, Four- day uterine weight assay, Abortifacient activity, Anti-implantation activity, Antigonadotrophic effect, Mating trial test, Anti-estogenic activity, Anti-ovulatory activity, Progestional activity using mice, rats and rabbits. In vitro methods such as Spermicidal activity, Estrogen receptor binding assay and gestagen receptor binding assay, androgen receptor binding assay.

KEYWORDS: Antifertility/Abortifacient, Contraception, Estrogen, Progesterone.

INTRODUCTION

Drugs which are used for preventing fertilization are called as antifertility agents. These are also known as oral contraceptive.[1] These drugs affects and are involved in the menstrual cycle and ovulation in females. Estrogen and progesterone are given in combined form as a birth control pills. The antifertility agents are consider as active in females when it prevents fertilization, ovulation, implantation and destroys the zygote and causes abortion. In males it prevents spermatogenesis, inhibits testosterone Or affects the gonadotropin of the organs or the mortality of the sperms.[2]

Overpopulation is becoming one of the major global problems causing much influence on economic, social and natural resources. It is of the serious problems in the developing countries. Moreover, increasing number of birth has got a deleterious effect on the health of mother and child. The increase in population is serious problem of developing world in the need for effective birth control measures.[3,4]

The regulation of human fertility has global effects in terms of resources depletion, population and poverty. Now it has become one of the priorities of the National Family Program and therefore, there is an urgent need to improve the access and the standard of contraceptive service in the country.[5]

Contraceptive agents are drugs used by women and men in the reproductive age group, to prevent unwanted pregnancies. This methods comprises all temporary and permanent measures to prevent pregnancy resulting from sexual intercourse.

Female sex hormones

Estrogen: Estrogens are a class of steroid hormones linked principally with the control of female sex organ responsiveness and of reproduction.[6] They are synthesized by the ovaries, the organ that produces the women's egg and placenta and in small amounts by the testis and adrenal cortex. The body makes three types of estrogens are 17-beta Estradiol (common in childbearing women), Estriol (the estrogen produced during pregnancy) and Estrone (the estrogen produced after menopause). The most potent biogenic form is 17-beta estradiol.[7]

Progesterone: Progesterone is the most important hormone in the female reproductive system. It is produced by the corpus luteum in the ovaries after ovulation and in the adrenal glands located near the kidney, as well as inside the placenta during the pregnancy,[8] Progesterone is essential for the implantation of the zygote and maintenance of early human pregnancy. The follicular phase of the menstrual cycle is estrogen dominated, while the luteal phase of the menstrual cycle is progesterone dominated.[9]

The synthetic analogue of progesterone is known as progestin. Progestins are oral contraceptives used to prevent pregnancy. It prevents the drop out of eggs from the ovaries and changing the cervical mucus and the lining of the uterus.[10]

Contraception

Contraception is the deliberate use of artificial techniques or methods such as various devices, sexual practices, chemicals, medicine or surgical procedures to prevent pregnancy. Any device or act whose purpose is to prevent a women from becoming a pregnant can be considered as a contraceptive. Contraceptive methods are supported a elementary understanding of the processes of successful reproduction, are not only important for individuals and families, but play an crucial role in population- regulation. Contraceptive methods include Oral contraceptive (OCs), Injectables, Intrauterine device (IUDs), Sterilization methods(male and female sterilization), Some barrier contraceptive methods like male and female condoms, provide advantageous protection against sexually transmitted diseases (STDs).

Evaluation of Antifertility agents

Female Antifertility agents are act by following mechanism: Inhibition of ovulation, prevention of fertilization, interference with the transport and implantation of fertilized ovum or destruction of early implanted embryo.

In vivo Methods

Sperm motility and count: Progressive motility was tested immediately. The right cauda epididymis was incised and seminal fluid was squeezed on a pre-warmed slide. 2 drops of warm 2.9% sodium citrate was added to semen and mixed by a cover-slip. The % of progressive sperm motility was evaluated visually at 400× magnification. Motility estimates were performed from 3 different fields in every sample. The mean of the 3 consecutive estimations was used because the final motility score. For spermatozoan count, the left cauda epididymis was incised and semen that oozed was quickly sucked into a red blood pipette to the 0.5 mark, and then diluted with warm normal saline up to the 101 mark. A drop of the semen mixture was placed on the Neubauer counting chamber and viewed underneath the magnification of ×40. The overall numbers of spermatozoan cells were counted and expressed as 10^6/mL.[11]

Vaginal opening: Vaginal opening is an apoptosis-mediated event used as an external index of puberty onset.[12] It occurs as a result of increasing estradiol secretion and might be stimulated by injection of estradiol into immature mice or rats.[13] Whereas vaginal opening within the rat occurs simultaneously with the first ovulation, vaginal opening within the mouse might occur up to ten days before the first vaginal cornification and therefore the onset of estrus cycle.[14] The age of vaginal opening in mice is documented by watching mice every morning from twenty four days to thirty days of age. Sometime the opening is detected through a simple visual examination of the vulva. In mice, vaginal opening occurs around twenty six days old.

Vaginal cornification assay: 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. If vaginal smears of rats show non-nucleated cornified epithelial cells, characteristic of estrus phase, it confirms the estrogenic nature of the test drug.[15,16]

Chick oviduct method: 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.[17]

Four- day Uterine Weight Assay: 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.[18]

Abortifacient activity: The plant extracts were tested in female albino rats for abortification activity. The vaginal smears of caged female rats of glorious fertility were monitored daily. Unstained material was observed under a light microscope. The proportion among the cells observed was used for the determination of the estrous cycle phases. Female rats were caged with males of proved fertility within the ratio of 2:1, in the evening of proestrous and examined the following day for the proof of sexual activity. Rats exhibiting thick clumps of spermatozoa in their vaginal smears were separated and which day was selected day 1 of pregnancy. These rats were indiscriminately distributed into 4 groups, a control group and 3 experimental groups of 6 animals every. Group I received vehicle only and served as control. Groups II, III, and IV received completely different extracts. On the 10th day of pregnancy the animals were

laparotomized beneath light ether anesthesia using sterile conditions. The two horns of uteri were examined to determine the implantation sites. Thereafter the abdominal wound was sutured in layers. Post operational care was taken to avoid any infection. The extract to be tested were then fed to operated pregnant rats, such that by an intragastric soft rubber tubing from day 11 up to the 15th day of pregnancy. The animals were allowed to travel to full term. Once delivery the pups were counted and therefore the antifertility activity of extract was evaluated. Litters were examined for any malformation.[19,20,21]

Percentage abortifacient activity = number of resorptions/number of corpus luteum × 100.[22]

Anti-implantation activity: Fertile female albino rats in estrous stage are made to mate with fertile male rats in ratio of 3:1. The vaginal smear from every 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 varied concentrations throughout 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 of rats are replaced back and abdominal incision is sutured in layers. The animals are restore in their cages and pregnancy is allowed to progress to complete the gestation. The number of litters delivered (if any) is counted.[18]

Antigonadotrophic effect: Female rats were studied for 5 consecutive normal estrus cycles by vaginal smear method. The rats were anaesthetized using ketamine (60 mg/kg) pretreated with atropine (1 mg/mL) and left side ovariectomy was performed. Left ovary was compound out rigorously from encompassing fatty tissue and dried by soaking on paper and weighed. The ovariectomized rats were divided into six groups and treated. On 12th day when treatment, the remaining right ovaries of all rats properly compound out victimisation same anesthetic condition. Cleaned, dried and their various weights were recorded and % increase in ovarian weight compared with weight of the left ovaries were calculated. % increase in the weights of ovary was calculated using the formula.[23]

% increase in ovarian weight = (weight of right ovary − weight of left ovary)/weight of left ovary × 100.

Mating trial test: The male rats was done mating trial test, 5 d before the termination of the experiment. Every male rat was cohabitated overnight with proestrous females in an exceedingly ratio of 1:2 and housed in an exceedingly single cage. Positive mating was confirmed by presence of spermatozoon and vaginal plug in the vaginal smear the subsequent morning. Every spermatozoon positive female was kept under observation and also the resultant pregnancies were noted, once dam gave birth. The subsequent reproductive parameters were then computed:

Mating success % = number mated/number paired × 10;

Fertility success % = number pregnant/number paired × 100;

Fertility index = number pregnant/number mated × 100.[11]

Antiestrogenic activity: All the Rats were ovariectomized and the weight of the ovaries were recorded. These ovariectomized rats were divided into thirty groups. The different doses of estradiol (0.1 mg/rat and 1.0 mg/rat) are given in all animals, except control group animals and followed by test compounds respectively for four consecutive days. The rats were anaesthetized by using ketamine (60 mg/kg, i.p.) on eleventh day and also the remaining right sided ovaries were dissected out from all the animals. The animals are well cleaned, dries and their several weights were recorded. The ovaries weight variations before and when treatment with extracts were calculated. % inhibition of ovarian weight was calculated using the following equation:

% inhibition in ovarian weight = [1−(XE−C)]/E−C × 100.[24]

Antiovulatory activity

HCG Induced Ovulation in Rats: This test is based on the principle that immature female albino rats don't exhibit spontaneous ovulation and administration of human chorionic gonadotropin (HCG) induces ovulation in 2 days. Immature female albino rats are given varied doses of the test drug, following which HCG is administered. The animals were sacrificed after two days of HCG administration; their ovaries are dissected and examined histologically. Compounds with antiovulatory activity inhibit the HCG induced ovulation.[18]

Cupric Acetate Induced Ovulation in Rabbits: 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 twenty one days to make sure that the rabbits are not pregnant. The test drug is administered and twenty four hours later, cupric acetate is given intravenously. Twenty four hours later administration of cupric acetate, 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.[18]

Progestational activity

Pregnancy maintenance test: 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.[17]

Clauberg/McPhail Test in Rabbits: 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. The last dose of drug administration 24 hours later, the animals are sacrificed and their uteri are dissected. Histological examination of the middle portion of uterine horn is completed for assessing the degree of mucous membrane 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.[17]

Carbonic anhydrase activity in Rabbits: It had been found that the quantity 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 set with estradiol and therefore the test drug or standard drug is given as in Clauberg test. After twenty four 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.[17]

In vitro methods

Spermicidal activity: It is a simple method and might be carried out in vitro directly on human seminal fluid. The fresh sample of human seminal fluid taken on slide is added with two drops of herbal drug extract in Sorensen phosphate buffer, mixed well and observed under microscope for motility of sperms. Immobility of sperms happens, in case of spermicidal herbal drug extract. An occlusion in vas deferens in male rat can occur due to granuloma type tissue formation there, resulting to male sterility. It will be assessed by carrying in vitro testing in male rats.[25]

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.[17]

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 androgenic hormone (testosterone) is employed as the standard. Inhibition of 5 α reductase – Compounds 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.[17]

CONCLUSION

Antifertility agents are those which are capable of preventing ovulation or fertilization and able to induce termination of pregnancy. The present review has provided detailed information about various in vivo and in vitro methods available for producing antifertility action in animal models.

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