



## Overview and Evaluation of Antifertility Models

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**Abstract :** The aim of this review is to provide comprehensive summary of antifertility definition, hormonal contraception, Drugs and Evaluation of Antifertility drug using various in vivo models. This paper emphasizes and reviews antifertility along with female hormones estrogens and progesterone's, which plays a very crucial role in hormonal balance in females. It also covers contraception specifically for antifertility treatment and methods of contraceptives ranging from combined Oral contraceptives (COC) and progesterone only contraceptives (POC). There are various advantages and disadvantages of using these contraceptives. This paper also covers methods of evaluating contraceptives such as Pearl Index and Life time Table analysis. The paper also reviews traditional Herbal drugs exclusively used for antifertility treatment. These drugs need to be explored further for toxicity assessment. This paper discusses the various methods of evaluation of antifertility agents and in vivo models using rats and mice. Antifertility Techniques such as estimation of sex hormones, Body and sex organs weight, Post coital antifertility activity (Pre-implantation activity), Histological analysis, Measurement of Biochemical and blood parameter, Determination of Serum cholesterol, and total proteins. This review creates a solid foundation upon which to further study the efficacy of plants that are both currently used by women as traditional antifertility medicines, but also could be efficacious as an antifertility agent with additional research and study.

**Keywords :** Antifertility agent; efficacy; medicinal plants, Hormonal Contraception.

### Introduction

Antifertility refers to the substances used to prevent or inhibit pregnancy. These substances ranges from natural agents present as herbal derivatives used from traditional methods to chemical synthetic moiety. They are also known as Oral Contraceptives.<sup>1</sup>

In today world scenario, population control is the need of hour. Every country has limited resources, which are depleting at fast rate. If population is not controlled, the quality of life is affected.<sup>2</sup>

Fertility regulation is certainly an essential pathway but not sufficient in itself to the optimization of population rise. A large variety of substances both natural and synthetic have been shown to intercept pregnancy either during the pre-implantation or post-implantation stages of pregnancy.

The antifertility substance is deemed to be active in females when it prevents fertilization, prevents ovulation, implantation, and destroys the zygote or causes abortion. In males, it prevents spermatogenesis, inhibits testosterone, or affects the gonadotrophin of the organs or the mortality of sperm.<sup>3</sup>

The development of hormonal contraceptives is one of the greatest scientific achievements of the 20th Century. About three decades back, some important discoveries were made which opened a new era in the

control of conception and fertility, viz: the discovery of the steroidal drugs, including steroidal contraceptives like estradiol and progesterone.

With the ever growing world population, contraception is an important health issue for the 21<sup>st</sup> century. Fertility is an issue of global and national public issues concerning the rapid growth of the country. The total world population of this century, the rate of increase of the population was about 10 million per year. Now it is increasing at a much faster rate of 100 million per year. If the rate of increase remains continuous at the same pace, it is expected to reach 7 billion by the end of the present century.<sup>4</sup>

The rapid increase of population has got an adverse effect on the international economy and as the increase is only limited to the developing countries, the problem becomes an acute one on the fruits of improvement in the different sectors, which are being eroded by the growing population. Moreover, increasing number of births has got a deleterious effect on the health of mother and child and hinders social and economic progress. The regulation of human fertility has global consequences in terms of resources depletion, population and poverty. Now, it has become one of the priorities of the National Family Programs and therefore, there is an urgent need to improve the access and the quality of contraceptive service in the country.<sup>5</sup>

Contraceptive methods are, by definition, preventive methods to help women to avoid unwanted pregnancies. This includes all temporary and permanent measures to prevent pregnancy resulting from coitus.

The international efforts to develop improved means of fertility control are based on the promise that the nature of available fertility control technology is highly important determinant for the success of planning programs.

## **Female Hormones**

### **Estrogens**

Estrogens are hormones that are important for sexual and reproductive development, mainly in women. They are also referred to as female sex hormones. The term "estrogen" refers to all of the chemically similar hormones in this group, which are estrone, estradiol (primary in women of reproductive age) and estriol. The body makes three types: Estradiol – Common in childbearing women, Estriol – The estrogen produced during pregnancy and Estrone – The estrogen produced after menopause. Estrogen is produced primarily in the ovaries, the organ that produces the woman's eggs. Once it is created, estrogen is transported to the body's tissues through the blood. During the menstrual cycle, estrogen levels change. In the middle of the cycle, estrogen levels are at their highest. Women will have lower estrogen levels during their periods. Because menstruation ceases in menopause, estrogen levels also drop at this time.<sup>6</sup>

### **Adverse Effects of Estrogens**

The use of estradiol in medications such as the contraceptive pill or hormone replacement therapy is associated with several adverse effects and some of these are -Abnormal vaginal bleeding, Painful periods or dysmenorrhea, Fibroid enlargement, Vaginal infections such as candidiasis, Changes in the amount of vaginal secretion, Cervical ectropion or cervical erosion/abrasion, Galactorrhea or the spontaneous flow of milk from the nipple when a woman is not nursing, Nipple discharge etc

### **Progesterone**

Progesterone is an essential element of a long and healthy life. The body's need for progesterone spans an entire lifetime, from being essential for conception to offering disease prevention and other significant health benefits throughout all life stages.

Progesterone effects a number of changes in the reproductive system and the mammary glands, which have important implications for its potential therapeutic use. Effects include

1. transition of the endometrium from the proliferative to the secretory phase.<sup>7</sup>
2. preparation of the endometrium for potential implantation<sup>8,9</sup>
3. maintaining pregnancy<sup>10</sup>

4. the reduction of uterine muscular activity through the development of increased numbers of  $\beta$ -adrenergic receptors and the reduction of sensitivity to oxytocin<sup>11,12,13</sup>
5. the reduction of prostaglandin synthesis<sup>14</sup>
6. stimulating the development of lobuli and mammary ducts in breast tissue<sup>15,16.</sup>
7. High levels of progesterone during pregnancy serve to prepare the mammary glands for lactation<sup>17.</sup>

In women, progesterone is secreted in the second half of the menstrual cycle (after ovulation) by the ovaries and, in much greater quantity, by the placenta during pregnancy. In both sexes, progesterone is also synthesized from cholesterol in the cortex of the adrenal gland, where it is a necessary precursor for the production of other hormones including testosterone, and it is also produced by cells in the nervous system. Progesterone's effects on a woman's body are far-reaching and affect her entire lifetime.

The progesterone hormone and its synthetic analog, known collectively as progestins, are not the same molecular structure and, therefore, do not behave the same way in the body. Progestins were developed because of a mistaken belief that bioidentical progesterone (i.e., progesterone that is biologically identical to that produced by the human body) could not be easily administered as an oral drug. Progesterone is essential for maintaining pregnancy, while the use of progestins during pregnancy is associated with fetal abnormalities.

Progesterone has both systemic and local effects. At the systemic level, progesterone increases diuresis through activation of the renin-angiotensin system, triggers the catabolic metabolism, relaxes smooth muscle cells, increases excretion of calcium and phosphorus, raises basal body temperature, has sedative and analgesic effects, improves visual memory and the proliferation and differentiation of osteoblasts and has additionally been found to have an immunosuppressive effect.<sup>18</sup>

This led to the use of synthetic gestagens such as derivatives of testosterone or 17-hydroxyprogesterone, as they are more resistant to hepatic degradation. But synthetic gestagens are associated with significant side effects due to their anti- or pro-estrogen effects, anti- or pro-androgen effects, mineralocorticoid effects and negative effects on glucose metabolism and lipid metabolism. Advances in micronization and the suspension of progesterone in oil solutions has led to new data, which has demonstrated the bioavailability of orally administered progesterone.<sup>19</sup>

## Contraceptives

Combined hormonal contraceptives consist of an estrogen and a progestogen, and act primarily by preventing ovulation through the inhibition of follicle-stimulating hormone and luteinizing hormone. The progestogen component also renders the cervical mucus relatively impenetrable to sperm and reduces the receptivity of the endometrium to implantation. These mechanisms render combined hormonal contraceptives very effective in the prevention of pregnancy. Annual failure rates vary between 0.02% (two per 10 000 women/year) when full adherence to instructions for use is assumed and 5% for typical use.<sup>20</sup>

## Methods of Contraceptives

Oral contraceptives (OCs), also known as "the pill", are the most popular method of contraception among female adolescents. The primary mechanism of action is inhibition of ovulation. In addition, oral contraceptives produce an endometrium that is not receptive to ovum implantation and cervical mucus that becomes thick and hostile to sperm transport. Tubal and endometrial motility are slowed.<sup>21</sup>

Typical use failure is directly related to patient compliance with use. Studies show that teens have a difficult time complying with daily use of OCs, therefore, alternative methods of contraception should be encouraged.

The progestin-only pill (the minipill) is less effective than combined oral contraceptives in preventing pregnancy. Patients using oral contraceptives (COC or POP) should receive counseling about and, as needed, prescriptions for emergency contraception (EC).<sup>21</sup>

## Oral Contraceptive Pill Types, Formulations, and Pill-Use Patterns

### A. There are two types of oral contraceptives:

1. Combined oral contraceptives (COCs), which contain an estrogen and a progestin
2. Progestin-only contraceptives (POPs), which contain a progestin but no estrogen. This pill is often referred to as “the minipill”

### B. Combined oral contraceptives are available in 2 basic formulations:

1. The monophasic formulation, in which each active pill contains the same doses of estrogen and progestin.
2. The multiphasic formulations can have varying amounts of estrogen and/or progestin in the active pills.

### C. There are multiple different patterns of combined oral contraceptive pill use that are options

1. **28-Day Cycling**– Most pill packs have 21 active hormone pills and 7 inactive (placebo) pills.
2. **Shortened pill-free interval** – Starting the new pack of pills on the first day of menstruation usually decreases the pill-free interval thus allowing less time for a new follicle to develop. Pill-free interval should not be more than 7 days.
3. **Extended regimens** – There is no biological reason to have monthly withdrawal bleeding on oral contraception. There are multiple extended regimens, and there are some pills that are formulated and packaged specifically for this type of extended regimen. If a client chooses an extended regimen, a monophasic, combined oral contraceptives must be used. Extended regimens in one form or another provide options for women who need to control the timing of their bleeding or have severe symptoms when bleeding. All clients using extended regimens have the potential for breakthrough bleeding and must be counseled as such.
  - a. **Bi-Cycling** – Skipping the placebo pills at the end of every other pack of pills yields one period after 6 weeks of active pills.
  - b. **Tri-Cycling** – Skipping the placebo pills at the end of 2 out of every 3 packs of pills yields one period after 9 weeks of active pills.
  - c. **Other Extended Regimens** (e.g. Seasonale) - COCs may be packaged by manufacturers as extended regimens.

## Benefits and Disadvantages of COCs and POCs

**A. Combined oral contraceptives (COCs) benefits** are Effectiveness, Safety in years of consecutive use without risk of complications, Ease of reversibility and Positive menstrual effects such as Decreased cramps, Decreased blood loss and Reduction of premenstrual symptoms.<sup>21</sup>

**B. Combined oral contraceptives (COCs) disadvantages:** Must be taken daily, Expensive, Provide no protection against sexually transmitted infections including HIV. Have possible side effects including Missed periods, Breakthrough bleeding, Nausea, Vomiting, Headaches, Depression and Decreased libido.

**C. Progestin-only contraceptive pill (POCs) benefits:** Estrogen-free, and therefore, useful for clients unable to tolerate the estrogen effects of combined oral contraceptives or who have contraindications against taking an estrogen-containing contraceptive, Can be taken during lactation and appears to have no harmful effect on blood pressure or on coagulation

**D. Progestin-only contraceptive pills (POCs) disadvantages:** Irregular menstruation Long Acting Hormonal methods

### Contraceptive Efficiency

A major problem in achieving effective contraception is patient compliance. To ensure success, hormonal, barrier, chemical or rhythm methods of contraception.

**Pearl Index:** It is the measurement of unplanned pregnancies even after the use contraceptive measures. A high Pearl index stands for a high chance of unintentionally getting pregnant; a low value for a low chance. The Pearl index will be determined by the number of unintentional pregnancies related to 100 women years.<sup>22</sup>

**Pearl Index :**  $\frac{\text{No. of Failures}}{100 \text{- women years of exposure}}$

**Life Table Analysis:** calculates a failure rate for each month of use.<sup>23</sup>

### Treatment

#### Traditional Medicinal Plants

In spite of availability of internationally accepted guidelines for the assessment of reproductive toxicity/antifertility potential of test substances, many published articles, on critical review, seem to lack reproducibility and are thus likely to mislead both the scientific community and the general public.<sup>24</sup>

**Table 1: Herbal Plants reported to have Antifertility Property**

Plant	Activity	Type	Dose/body weight (mg/kg)
<i>Terminalia bellirica</i>	Antifertility effect	Alcoholic extracts	50 mg/d
<i>Quassiaamara</i>	Antifertility effect	Chloroform extracts	Single daily intramuscular injections of the extract for 15 d
<i>Ocimum sanctum</i>	Antifertility property	Benzene extract	300
<i>Juniperusphoenica</i>	Antifertility property	Ethanol extract	Intraperitoneal injections of 400
<i>Abrusprecatorius Linn</i>	Antifertility property	70% methanolic extract	20 and 40
<i>Aegle marmelos</i>	Antifertility property	50% ethanolic extract	100, 200, and 300
<i>Albizialebeck</i>	Antifertility property	Methanolic extract	50, 100, and 200

### Evaluation of antifertility agents

The therapeutic value, efficacy and toxicity of drug may be evaluated in animals experimentally, followed by clinical trials. In-vivo animal models are employed to assess anti-fertility activity in experimental animals like rat and mice.

For the study of anti-fertility activity many in-vivo models have been used.

#### Estimation of sex hormones

Blood samples were collected from rats for estimations of serum levels of sex hormones. Sera were separated into clean bottles, stored frozen and used within 12 h of preparation for the estimation of testosterone, estrogen level, prolactin, follicle-stimulating hormone (FSH) and luteinizing hormone (LH).<sup>25</sup>

#### Assessment of sperm motility and count

Progressive motility was tested immediately. The right caudaepididymis was incised and semen was squeezed on a pre-warmed slide. Two drops of warm 2.9% sodium citrate was added to semen and mixed by a cover-slip. The percentage of progressive sperm motility was evaluated visually at 400×magnification. Motility estimates were performed from three different fields in each sample. The mean of the three successive

estimations was used as the final motility score. For sperm 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 under the magnification of  $\times 40$ . The total numbers of sperm cells were counted and expressed as  $10^6/\text{mL}$ .<sup>25</sup>

### Assessment of sperm viability and morphology

A viability study (percentage of live spermatozoa) was done using eosin/nigrosin stain. A drop of semen was squeezed onto a microscope slide and two drops of the stain were added. Thin smears were then prepared and observed under a light microscope at  $\times 400$  magnification. Viable sperm remained colorless while non-viable sperm stained red. The stained and the unstained sperm cells were counted using  $\times 40$  microscope objectives and an average value for each was recorded from which percentage viability was calculated. To determine the percentage of morphologically abnormal spermatozoa, the slides stained with eosin nigrosin (5 slides/rat) viewed under a light microscope at  $400\times$  magnifications. A total of 300 sperm cells was examined on each slide (1500 cells for each rat), and the head, tail and total abnormality rates of spermatozoa were expressed as a percent.<sup>25</sup>

### Mating trial test

Mating trial test of male rats was done, 5 d before the termination of the experiment. Each male rat was cohabitated overnight with proestrous females in a ratio of 1:2 and housed in a single cage. Positive mating was confirmed by presence of sperm and vaginal plug in the vaginal smear the following morning. Each sperm positive female was kept under observation and the resultant pregnancies were noted, when dam gave birth. The following reproductive parameters were then computed<sup>25</sup>:

Mating success % = number mated/number paired  $\times 10$ ;

Fertility success % = number pregnant/number paired  $\times 100$ ;

Fertility index = number pregnant/number mated  $\times 100$

### Body and sex organ weights

The initial and final body weights of the animals were recorded. The testes, epididymides, seminal vesicle and ventral prostate were dissected out, freed from adherent tissues and blood, and weighed to the nearest milligram. Organ weights were reported as relative weights (organ weight/bodyweight  $\times 100$ )<sup>25</sup>

### Quantification of fructose in seminal vesicle

For fructose quantification, seminal vesicular homogenate was prepared at a tissue concentration of 50 mg/mL. The supernatant (seminal plasma) was deproteinized by adding 50 mL of zinc sulfate and sodium hydroxide to make a total dilution of seminal plasma 1:16, followed by centrifugation at 2500 r/min for 15 min.

For fructose measurement, 200 mL of clear seminal plasma was used and the optical density of standard and samples were measured against blank at 470 nm. The concentration of fructose was obtained by plotting the value in standard curve and the value expressed in the unit of mmol/mL of seminal plasma.<sup>25</sup>

### Post-coital antifertility activity (Pre-implantation activity)

The anti-implantation activity is expressed as the percentage of animals showing absence of implantations in uteri when laparotomised on day 10 of pregnancy. Vaginal smears from each rat were monitored daily and the rats with normal estrous cycle were selected. Rats found in proestrus phase of cycle were caged with males of proven fertility, in the ratio 2:1 and examined the following morning for evidence of copulation. Rats exhibiting thick clumps of spermatozoa in their vaginal smears were separated and that day was designated as day 1 of pregnancy and those rats were divided into five groups containing six rats in each group. The extract was administered from day 1–7 of pregnancy. The powdered drug was also administered from day 1–7 of pregnancy. Control rats received the vehicle (distilled water). On day 25, laparotomy was performed under light ether anesthesia and semisterile conditions. The uteri were examined to determine the number of implantation sites and no of corpora lutea graviditatis<sup>26,27,28,29</sup>

Frequency of pre-implantation losses =  $\frac{\text{missing no. of implants (corpora lutea implants)}}{\text{no.of corpora lutea}} \times 100$

### Effect on estrous cycle

The female animals were artificially brought into estrus phase (heat) by administering either suspension of ethinyl estradiol orally at the dose of 100 mg/animal 48 h prior to the pairing and subcutaneous administration of progesterone at the dose of 1 mg/animal 6 h before the experiment or alternatively by the sequential administration of estradiol benzoate (10 mg/100 g body weight) and progesterone (0.5 mg/100 g body weight) through subcutaneous injections, 48 and 4 h respectively. Estrous cycle was determined between 8 am and 10 am using vaginal smear method. Vaginal secretion was collected with a plastic pipette with 10 mL of normal saline. The vagina was flushed three times with the pipette and the vaginal fluid was placed on a glass slide. Different slides were used for each animal. The unstained secretion was observed under a light microscope. After confirmation of regular four day cycle for 2 weeks the animals were selected for study and divided into six groups and treated with test drugs. The effect of test drugs on the estrous cycle was monitored for 28 d.<sup>26,30</sup>

### Anti-estrogenic activity

All the rats were ovariectomized by the same methods described in previous procedure and the weight of the ovaries were recorded. The ovariectomized rats were randomly taken and divided in thirteen groups. Except control, other groups were administered with different doses of estradiol (0.1 mg/rat and 1.0 mg/rat) and followed by test compounds respectively for 4 consecutive days. On eleventh day, the rats were anaesthetized using ketamine (60 mg/kg, i.p.) and the remaining right sided ovaries were dissected out from all the animals. Properly cleaned, dried and their respective weights were recorded. The ovaries weight variations prior to and after treatment with extracts were calculated. Percentage inhibition of ovarian weight was calculated using the following equation:

Percentage inhibition in ovarian weight =  $[1 - (XE - C)] / E - C \times 100$ .

Where, C indicates mean ovarian weight from rats treated with vehicle, E for estradiol and XE indicates the mean ovarian weight of rats treated with extract and estradiol.<sup>26</sup>

### 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 dissected out carefully from surrounding fatty tissue and dried by soaking on filter paper and weighed. The ovariectomized rats were divided into six groups and treated. On 12th day after treatment, the remaining right ovaries of all rats properly dissected out using same anesthetic condition. Cleaned, dried and their respective weights were recorded and percentage increase in ovarian weight compared with weight of the left ovaries were calculated. Percentage increase in the weights of ovary was calculated using the formula.<sup>26</sup>

Percentage increase in ovarian weight =  $(\text{weight of right ovary} - \text{weight of left ovary}) / \text{weight of left ovary} \times 100$

### Histological analysis

Testes and uteri were carefully dissected out following abdominal incision from male and female rats respectively and fixed in 10% normal-saline and processed routinely for paraffin embedding. Sections of 5 mm from both were obtained with rotary microtome, stained with Hematoxylin and Eosin Stalin (H/E) respectively and observed under a light microscope.<sup>31</sup>

### Measurement of some biochemical and blood parameters

Blood samples were collected from the heart of each rat at the time of scarification into non-heparinized and heparinized tubes. Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphates, creatinine and urea in addition to red blood cell (RBC) count, total leucocytic count (TLC),

hemoglobin (Hb) concentration, packed cell volume (PCV), cholesterol and total proteins were determined by standard methods.<sup>32</sup>

#### **Determination of testicular and serum cholesterol (Chod PAP method)**

Cholesterol is the precursor in the synthesis of many physiologically important steroids such as bile acids, steroid hormones and vitamin D and its requirement for normal sexual activity has been well established. Testicular and serum cholesterol concentrations may be determined by the Chod-PAP method as briefly, 0.02 cm<sup>3</sup> of the working reagent and the absorbance of the resulting mixture is read after 5 min at 546 nm.<sup>33</sup>

#### **Determination of total protein**

A timed rate biuret method was used to measure the concentration of total protein in serum or plasma. Proteins in the sample combined with the reagent producing alkaline copper-protein chelate. The rate change in absorbance was monitored by a detector at 545 nm. The observed rate of chelate formation is directly proportional to the total protein concentration in the sample.

#### **Conclusion**

In conclusion, this review aims to summarize antifertility and Evaluation of antifertility activities. Already, medicinal plants have a proven efficacy as antifertility agents. The mechanism of action of traditional drugs reported to have antifertility activity need to assess further for toxicity studies. Further research is required to ascertain the antifertility activity of novel Herbal and tradition drugs to exploit their activity as antifertility agents. Comprehensive Assessment utilizing in vivo animal models using rats and mice shall further strengthen their roles.

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