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"New Animal Models for Antifertility Agents: A Comprehensive Review"

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Abstract

This review focuses on the development of innovative animal models for evaluating antifertility agents, highlighting their importance in understanding the efficacy and safety of new contraceptive methods. These models are essential for studying the pharmacodynamics and pharmacokinetics of potential antifertility treatments. The review addresses the challenges in developing these models, including interspecies differences and ethical issues. There is a growing need for contraceptives that are effective, user-friendly, reversible, and culturally acceptable, as current methods often fall short in meeting diverse needs. Research has explored various contraceptive methods such as hormonal pills, injectable, intrauterine devices, and implants, each presenting specific benefits and limitations. Animal models provide a valuable controlled environment for testing these methods, offering insights that are not always possible in human trials. However, translating results from animal models to human applications remains challenging due to physiological differences between species. The review underscores the importance of interdisciplinary collaboration and ethical research practices in overcoming these challenges and advancing global reproductive health.

Keywords: Antifertility agents, Animal models, Contraceptive research, Reproductive health, Hormonal contraceptives, Intrauterine devices

Introduction

Novel approaches to contraception involve the utilization of anti-fertility agents, which disrupt fundamental reproduction processes in both genders, thereby impeding fertility. The essentials for advancing innovative models, stringent regulatory frameworks for efficacious agents, and targeted research potential are particularly pronounced in developing nations. In the quest for an ideal contraceptive, the pinnacle would be a method that ensures 100% effectiveness, user-friendliness, and reversibility. It should be universally acceptable. The intervention would be culturally appropriate, taking into account local norms and beliefs regarding sexuality, reproduction, menstruation and gender roles. Also, it would be usable across diverse groups with varying health conditions [1]. A diverse range of contraceptive options is essential to accommodate the diverse needs of individuals at various stages of their lives. One approach may be optimal for young individuals or those who engage in infrequent sexual activity. An alternative approach may be more appropriate for young women who currently breastfeeding and desire to regulate the timing between their pregnancies. Another approach that may be optimal for older couples who do not want any more children but are not interested in sterilization is available. However, existing contraceptive options do not adequately cater to the needs of many individuals [2]. In contrast to typical products, we usually rely on the interactions between industry, the market, and government policies to produce a diverse array of choices and drive the pace of product development. However, contraceptives deviate from this norm due to distinct factors. Government regulations have restricted both the quantity and diversity of contraceptive options accessible to consumers, as well as impeding the speed at which new contraceptive methods are developed. This circumstance arises from the unique attributes of modern contraceptive techniques and our approach to them [2].

Contraceptive practice

The significant advancements of the 20th century have greatly improved our capability to regulate fertility. The vast numbers of adults in the US, as well as hundreds of millions worldwide, have utilized contraceptive methods. In the United States alone, among the 54 million women aged 15 to 44 who have engaged in sexual intercourse, 95% have employed contraception at some point [3]. Above 70% of American married women of reproductive age group and parents use contraception. The pill became the most popular contraceptive in 1987, which was followed by female sterilization, condoms and vasectomies [4]. Never before has a systemic medicine like oral contraceptive been used so extensively by mostly healthy women for prevention [2].

Before discussing the potential benefits of novel contraceptive methods and models used, it is valuable to examine the existing array of contraceptives. Table 1 offers a comprehensive overview of contraceptive methods currently accessible in the market, detailing their prevalence of use, failure rates, and a summary of their primary advantages and disadvantages. It's crucial to understand that failure rates, which encompass the rate of unintended pregnancy within the 1st year of use, account for both user-related failures—such as improper or inconsistent usage—and inherent method failures.

Method	Estimated Use	% Accidental Pregnancy in the 1st Year	How It Works	Advantages	Disadvantages	Reference
Male Condoms	21.8%	13%	A thin sheath is worn over the penis to collect semen during ejaculation, preventing sperm from entering the vagina	Easily accessible, protects against STIs	Requires consistent and correct usage, may reduce sensitivity	[5]
Female Condoms	0.6%	21%	The pouch that covers the cervix and lines the walls to block sperm from accessing the egg	Provides control to the receptive partner	Requires correct insertion, it may be difficult to trace	[5]
Oral Contraceptives	12.6%	7%	Hormonal pills prevent sperm from fertilising eggs by blocking ovulation and thickening cervical mucus	Highly effective when used correctly	Requires daily adherence, may have side effects	[6]
Injectable Contraceptives	3.2%	4%	Hormonal injections are administered every few months to prevent ovulation and alter cervical mucus, impeding sperm movement	Long-lasting, does not require daily attention	May cause irregular bleeding, and cannot be immediately reversed	[7]
Intrauterine Devices (IUDs)	14.3%	0.1%	T-shaped device planted in the uterus, releasing copper to prevent fertilization and implantation	Highly effective and long-lasting	Requires insertion by a healthcare provider, may cause cramping	[8]
Implants	2.6%	0.05%	Subdermal rods placed in the	Highly effective and	Requires minor surgical	[9]

			upper arm slowly administer hormones that increase the viscosity of cervical mucus and suppress ovulation	long-lasting	procedure for insertion and removal	
Diaphragm	0.2%	12%	A soft cup is Placed into the vagina. it covers the cervix to prevent fertilization of an egg by sperm	Provides some control to the user	Requires correct fitting and use with spermicide	[10]
Cervical Cap	0.2%	14%	A small, dome-shaped device is placed to cover the cervix and block the entry to the uterus	May be placed in advance	Requires correct fitting, may increase risk of urinary tract infection	[11]
Tubal Ligation	18.6%	0.5%	fallopian tubes sealed to prevent entry of sperm to reach the egg	Permanent method	Requires surgery, irreversible	[12]
Vasectomy	8.4%	0.15%	Surgery blocking the vas deferens to prevent the release of sperm during ejaculation	Permanent method	Requires surgery, irreversible	[13]

Recent years have seen a growing interest in antifertility research, driven by the need to meet diverse contraceptive needs globally. Researchers are increasingly using animal models to understand fertility regulation and assess the efficacy and safety of new contraceptive agents. This review highlights recent advances in animal models used in antifertility research, synthesizing findings from various studies to provide insights into the interaction between contraceptive agents and reproductive physiology. It underscores the importance of animal models in developing and refining contraceptive strategies, pointing to future research directions and potential clinical applications. As societal attitudes towards family planning evolve, exploring innovative contraceptive methods remains crucial [14]. This review aims to

inspire further research in this vital area, ultimately aiding the development of safer, more effective, and accessible contraceptive options worldwide.

Methods for female contraception [15]

Possible modes of action for female anti-fertility agents include:

1. Suppression of ovulation
2. Inhibition of fertilization
3. Disruption of the early implanted embryo
4. Disruption of the attachment of a fertilized egg to the uterine lining
5. Disruption of attachment of an egg to the uterine lining

Anti-ovulatory Activity

Ovulation Induced by Human chorionic gonadotropin (HCG) in Rats

Young female albino rats do not exhibit cyclic changes in the vaginal epithelium or ovulate spontaneously. 2 days after follicular maturation is induced by priming with Human chorionic gonadotropin (HCG), spontaneous ovulation follows. Anti-ovulatory medication injections done before the induction process will stop ovulation. We screen anti-ovulatory drugs using this idea [15].

Procedure: Mature albino rats of 24 and 26 days old are employed. Different test medicines are given to the animals at varying dosages. The procedure of exogenous infusion of Human chorionic gonadotropin (HCG) followed by test medication to induce ovulation. Animals are killed 2 days later; the ovaries are removed, stored in 10% buffered formalin, and examined histologically to make a comparison with control groups [16].

Cupric Acetate-induced Ovulation in Rabbits

Rabbits are reflex ovulators: They experience ovulation shortly after encountering men, receiving mechanical stimulation of the vagina, or being exposed to specific chemicals such as cupric acetate. Cupric acetate is employed in these techniques to stimulate ovulation. The rabbit's ovulation occurs shortly after the administration of cupric acetate (0.3mg/kg iv of 1% cupric acetate in 0.9% saline). Administering anti-ovulation medication 24 hours before the induction techniques effectively inhibit ovulation [17].

Procedure: The experiment uses 3-4 kg female rabbits that are sexually mature. To be sure they are not pregnant and to avoid mating-inducing ovulation, animals are housed in isolation for a minimum of 21 days. They are given a test medication and then cupric acetate a day later. 1 day or 2 days after the rabbits are killed, the ovaries are inspected. Each animal has their total number of ovulation sites on both ovaries noted. The uterus and ovaries are then removed, stored in 10% buffered formalin, and examined histologically [18]. **Fig 1 [19]**

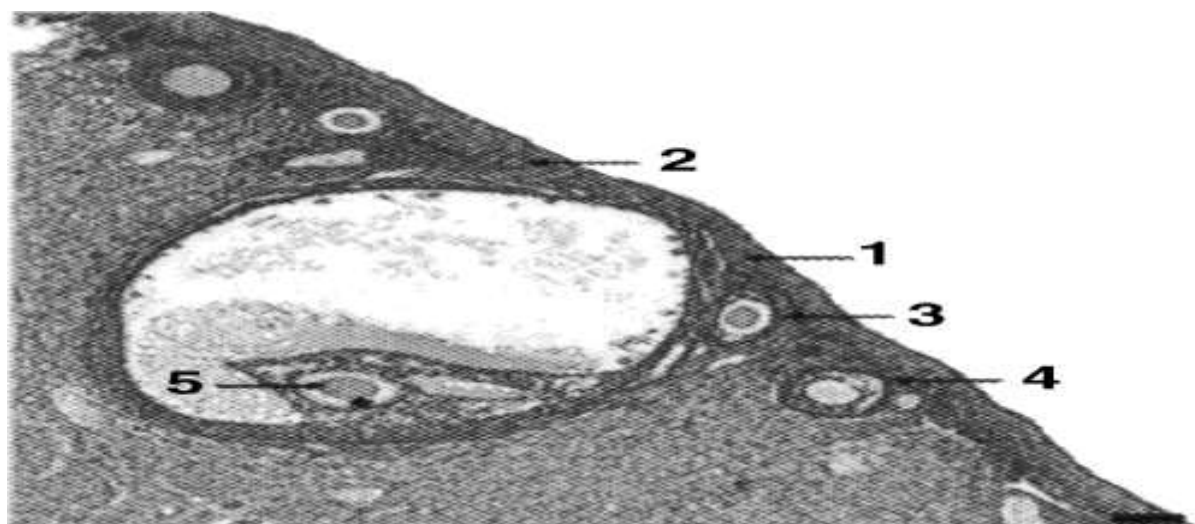


Figure 1 displays the histological structure of a rabbit ovary, illustrating the various stages of follicular maturation observed in pregnant rabbits. These stages encompass the presence of primary oocytes within primordial follicles (1), secondary oocytes located in primary (2), secondary (3), antral (4), and Graafian follicles (5). Additionally, the depiction highlights the zona pellucida surrounding oocytes in secondary and Graafian follicles. Staining of histological sections is done with hematoxylin-eosin, with a scale bar provided for reference, indicating a length of 100 micrometres [19].

Estrogenic Activity

Estrogen is mostly used as contraception and it is justified by the idea that too much exogenous estrogen suppresses FSH and LH, therefore inhibiting ovulation

In Vivo Methods

Vaginal opening

The underlying idea of this test is that estrogenic substance causes immature female albino mice and rats to open their vaginas. Estrogenic action is observed as the sign of the entire vaginal opening.

Procedure: Immature female mice and rats 18-day-old, and 21-day-old are used for the study. Both drugs are administered intramuscularly in cottonseed oil. The time of complete vaginal opening can be observed as a sign of activity [20].

Assay of Water Uptake

Its principle is based on the uterus's response to estrogen by uptake and increasing the level of water. A peak is seen after 6 hours of administration [20].

Procedure: Ovariectomized mice and rats may be used, because this essay employs the uterine weight increase as the response, the uterus must remain intact during ovariectomy. Immature 18-day-old mice or young, 20-day-old rats received from the breeder not more than two days before the experiment started are suitable. The groups are also made at random. The

group which was under controlled was injected with 0.1 ml of cotton seed oil subcutaneously. —The control group receives estrogen at a dose within the range of 0.01 to 0.1 μm as it makes it possible to perform further establishing of dose-response curve; The animals die of cervical dislocation 5 hours after drug administration, and the uteri are promptly removed. A longitudinal incision made through the outer layer of the abdomen starts the procedure. The uterus is removed using a clip or forceps and cut away from the vagina. Cut closer to the ovary, the uterine horns are then separated from the connective tissues. Placed on dry filter paper and covered with moist filter paper, the uteri are maintained moist [21].

Subsequently weighed on a sensitive balance. Reword the uteri which was dried at 100°C in an oven, for 24 hours. The increase in the percentage of water values is calculated and compared with another group [21].

Ovariectomy: Animal slightly Anaesthetized with ether. A cut is made in the posterior region. By altering position, the incision can be easily moved from one side to the other. A small incision is made directly at the region of the ovary through the abdominal wall and surrounded by a layer of adipose tissue. A set of forceps is used to carefully hold the adipose tissue around the ovary, ensuring that the covering that protects the ovary is not damaged. The tip of the uterine horn is subsequently pressed using forceps, and the ovary along with the fallopian tube is removed through a single incision made by a pair of fine scissors. Typically, there is no presence of blood. The absorbable sutures are used for muscular wounds, while the outer wound of the skin is sutured with nylon sutures.

Four-day Uterine Weight Assay

It is based on the fact that estrogen stimulates protein synthesis because it will ultimately increase uterine weight. It shows a peak in uterine weight at about 40 hrs [20].

Procedure: The test medication can be administered intramuscularly in cottonseed oil to immature or adult ovariectomized Albino mice or rats for three consecutive days. On the fourth day, animals are euthanized by cervical fracture, the uteri are promptly removed, and the uterine content is delicately expelled (accurate results require the removal of uterine contents). The uteri are promptly weighed while still moist. To determine the increase in dry weight, the uteri can be dehydrated in an oven at a temperature of 100°C for 24 hours, after which they can be weighed again. A sigmoid curve is generated when plotting the log dose against the wet weight. This curve allows for the determination of the ED 50, which may be used to compare the test substance with estradiol [21].

Vaginal Cornification

Based on the observation of cyclic vaginal qualification in guinea pigs by *Stockard and Papanicolaou (1917)* [22], *the Allen- Doisy (1923)* [23], *FORD, D. H., & YOUNG, W. C. (1951)* [24] found the vaginal cornification in rodents. An alternative method for obtaining matured oocytes is through in vitro maturation (IVM) instead of traditional superovulation protocols. Epidermal Growth Factor (EGF) and Insulin-like growth factor I (IGF-I). EGF, a 6.2 kDa peptide produced in the ovary and fenestrated endothelial cells of vascular antral

follicles etc. Despite the guinea pig's significance in reproductive research, IVM utilization remains limited in this species [25].

This essay is centre around the observation that rats and mice undergo cyclical ovulation, which is accompanied by hormonal fluctuations that result in alterations in the vaginal epithelial cells. The estrus cycle is categorized into four distinct phases: proestrus, estrus, met estrus, and diestrus. Drugs exhibiting estrogenic action induce an estrus cycle in animals.

Procedure: The study utilizes adult female albino rats that exhibit normal estrous cycles. Animals undergo a variety of tests and are administered regular medications. The vaginal changes can be assessed by collecting vaginal smears and analyzing them for the presence of cornified cells, leukocytes, and epithelial cells in both the control and treated animals, twice a day for 4 days. The medicine, which induces animals to directly enter the estrus stage while bypassing previous stages, is regarded as having estrogenic activity [26].

Vaginal Smears

In order to track the advancement of the estrous cycle, vaginal smears were obtained twice a day, once in the morning (at 9 AM) and once in the afternoon (at 5 PM), over a period of 20 days. To accurately classify each phase, daily sampling intervals of 8 hours were maintained, taking into account the brief duration of estrous phases. Vaginal samples were collected using sterile cotton-tipped swabs that were dampened with distilled water. The swabs were inserted to a shallow depth of roughly 1 cm to avoid undue stimulation of the cervix and the possibility of pseudo-pregnancy. The vaginal epithelial cells were gathered, placed onto glass slides, allowed to dry in the air, and then preserved in a solution of ethanol and ether. The Shorr method [27] was utilized for staining, which distinguishes epithelial cells based on their cytoplasmic coloration. Acidophilic cells are stained dark orange, while basophilic cells are stained blue, thereby facilitating their identification. Afterwards, the smears were dehydrated, stained with Shorr stain, dehydrated further, made transparent using xylene, mounted under coverslips, and observed using an Olympus photomicroscope [28].

Morphometric analysis

Analyzed vaginal smears were observed using a light microscope to ascertain the number of different types of epithelial cells present throughout each stage of the estrous cycle. An estrous cycle is defined as the period between one phase of estrus and the following phase of estrus. The categorization of vaginal smears relied on the shape and staining properties of epithelial cells, along with the presence or absence of leukocytes and mucus. The morphometric study involved classifying epithelial cells based on their size and staining properties, specifically into small or big basophilic cells, nucleated or enucleated acidophilic cells, and pre-acidophilic cells. The frequency of each cell type was quantified and shown as a percentage, with individual scores produced for each rat slide, irrespective of the evaluated phase [28].

The frequency of each type of epithelial cell was determined by dividing the average number of cells by the total number of slides in the corresponding phase, and expressing the cell

frequency as a percentage. The mean leukocyte count was evaluated using a scale that ranged from 1+ to 4+, representing different leukocyte counts per field. The Leica QWin V3 image analysis system was used to measure the major axis of several cell types in order to determine their diameter. Each defined phase was analyzed by measuring ten cells of each kind per slide.

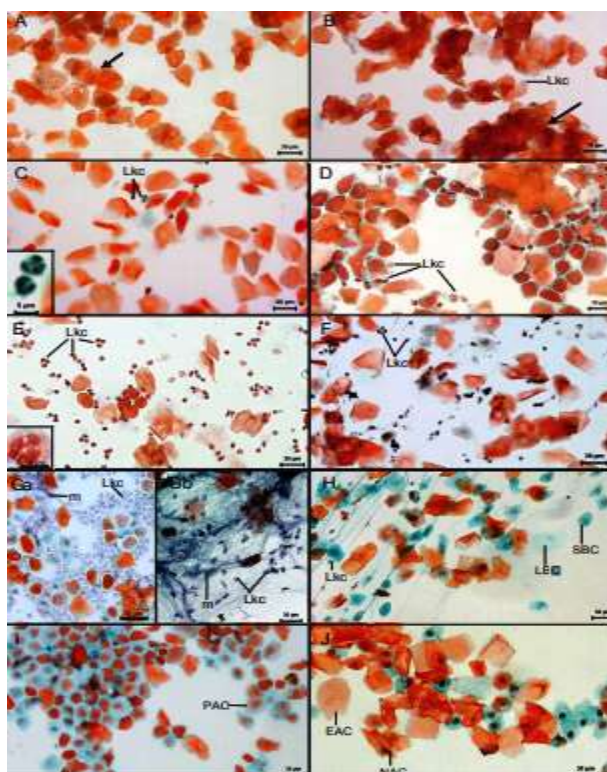


Figure 2 illustrates vaginal smears stained using the Shorr method across different phases and transitional periods of the estrous cycle. A depicts estrus, showing clumps of enucleated acidophilic cells. In B, transitioning from estrus to metestrus I, clumps and leukocytes are visible. Metestrus I (C) also exhibits leukocytes. The transition from metestrus I to metestrus II is depicted in D. Metestrus II (E) displays numerous leukocytes. Transitioning from metestrus II to diestrus is shown in F. G demonstrates mucus stained in blue during early diestrus, with leukocytes trapped within abundant mucus during mid-diestrus. Transitioning from diestrus to proestrus is illustrated in H, featuring a small basophilic cell and a large basophilic cell. Proestrus (I) showcases pre-acidophilic cells. Lastly, J shows the transition from proestrus to estrus, where nucleated and enucleated acidophilic cells are present, alongside leukocytes indicated as "Lkc" in the images [28].

In-Vitro Methods

Estrogenic-Receptor-binding Assay

The estrogenic receptor binding assay is based on the principle of competitive binding between labelled estrogenic receptors and unlabelled estrogenic receptors. In the estrogen receptor, estrogenic chemicals displace an escalating dose of the label estrogen in a titer-dependent manner [26].

Procedure: Cytosol preparation: the uteri from 18-day-old female albino mice are removed and ground in a conical homogenizer at 0°C using Todd Rodnstein buffer at 1:50 (w/v) ratio. The human endometrium from postmenopausal women was fast frozen within 2 h of the hysterectomy and then stored in liquid nitrogen as described above. The frozen endometrium was crushed and thoroughly mixed with a Tris-Sucrose buffer 1:5 (weight/volume) ascribing to the establishment. [16 h] Homogenates are centrifuged at 105,000 g for 1 hour. Number of selective attachments in the cytosol of mouse uterus; concentration based on steroid, duration for incubation and temperature. Three 125 ml measures of equivalent volumes of cytosol are incubated with either 5 or 25 nM labelled steroid. Incubation is for 2 or 24 hr at 0°C; on occasion, incubations are also performed for 2 to 5 hours at room temperature. Incubation is done both in the absence of (total binding) and the presence of a 100-fold excess of inert steroid (nonspecific binding). Using the method of Dextran-coated charcoal (DCC) adsorption, the strength of the steroid is determined.

Polyphenylene Carboxymethylene (PPCM) in Yaso-GEL Shows Potent Antimicrobial Activity Against Neisseria Gonorrhoeae in Preclinical Studies [27]

PPCM was a condensation polymer with contraceptive and antibacterial capabilities. "It has shown efficacy in pre-clinical studies at inhibiting a range of human disease-causing sexually transmitted viruses, including HIV, herpes simplex virus, SARS-CoV2 and the Ebola" IANS Yaso-GEL is a vaginal gel formulation with a safe API. The present study investigated the effectiveness of PPCM against a common agent of gonorrhea, which was *Neisseria gonorrhoeae* and carried out through in vitro as well as in vivo experiments. Using both an agar dilution and microtiter plate-based method, we established MIC values of PPCM for 11 strains of *N. gonorrhea* as shown in Table (1). These experiments resulted minimum inhibitory concentration spectrum from 5–100 µg ml⁻¹ (agar dilution method) and 50-200 µg/mL⁻¹¹ for mushroom extracts by microtiter plate method. In a mouse model of *N. gonorrhea* genital tract infection, the effectiveness of PPCM in a hydroxyethyl cellulose (HEC) gel and Yaso-GEL with PPCM was evaluated by applying them vaginally before the bacterial challenge and assessing the presence of bacteria in vaginal swabs over five days. The findings demonstrated that the prevention of infection was directly proportional to the concentration, as Yaso-GEL with 4% PPCM effectively prevented infection in all animals. Further analysis suggested that PPCM compromises bacterial membrane integrity, which could be the underlying mechanism for its antimicrobial effect. These findings suggest that Yaso-GEL, with its contraceptive and antimicrobial properties, could be a promising multipurpose prevention technology to address both unintended pregnancies and sexually transmitted infections (STIs), particularly gonorrhoea. This product could be highly beneficial for women across various socio-economic, cultural, and geographical backgrounds, providing a non-hormonal, non-systemic means of prevention [27].

One study Todorov, S. D et al (2023) [30] explored the antimicrobial properties of bacteriocin-like inhibitory substances (BLIS) produced by *Enterococcus mundtii* CRL35 and *Enterococcus faecium* ST88Ch, focusing on their activity against *Candida albicans* 1281. A total of 75 strains of lactic acid bacteria (LAB) were screened for anti-*Candida* activity, with *E. mundtii* CRL35 and *E. faecium* ST88Ch exhibiting significant inhibitory effects [28, 29]. The antimicrobial activity of BLIS from these strains was measured in arbitrary units (AU) per milliliter, with *E. mundtii* CRL35 showing an increase from 6,400 AU/mL to 25,600 AU/mL after ammonium sulfate precipitation and SepPakC18 hydrophobic chromatography, while *E. faecium* ST88Ch maintained activity at 800 AU/mL [30].

Both strains were non-hemolytic, meaning they had a low potential to harm red blood cells. In this sense, the co-aggregation results obtained in collated assays presented a considerable and representative profile of *E. mundtii* CRL35 with *E. faecium* ST88Ch and also with *C. albicans* 1281 showing interesting results for the potentiality of antimicrobial activity together. Adsorption studies showed that temperature, pH and the presence of contraceptive Nordette-28 affected the adsorption of BLIS to *C. albicans* 1281. The adsorption was found to be optimal at 37°C and higher pH. —Nordette-28 improves adsorption of BLIS, especially at pH 5.0.

Similarly, the influence on *E. mundtii* CRL35 and *E. faecium* ST88Ch growth by several antibiotics and other types of medications, such as contraceptives was determined. Most of the antibiotics displayed a relatively lower rate of resistance; however, none of the contraceptives were patterning inhibition against these strains. Finally, *C. albicans* 1281 cells treated with BLIS displayed a dose-dependent killing effect; *E. mundtii* CRL35 exhibited greater activity at higher concentrations determined through the percentage of relative cell lysis that was equivalent to 79.35%, while *E. faecium* ST88Ch reduced bioactivity "The current study concludes that LAB-derived BLIS can be effectively designed as an antimicrobial agent for the future potential therapy and development of new products against *C. albicans*," the researchers said [30].

Progestational Activity

In Vivo Methods

Pregnancy Maintenance

Progesterone is most important for — (A) The maintenance of pregnancy (B) Ovulation (C) Milk secretion (D) the Development of low voice (centralising puberty). It is on this principle that the assay for progestational chemicals was based [26].

Procedure: Ovaries are cut out from different groups of pregnant rats either on the fifth day or the tenth day or fifteenth day of the pregnancy. The animals were subjected to multiple tests with normal medications 'Pregnant' rats are then euthanized after 5, 10 or 15 days. After the experiment, the average number of viable foetuses in standard irradiated and excised groups of regularly treated experimental models was compared with that in radiant only

control group (not undergone ovariectomy). The effective dose (ED50) of progesterone in rats is 5 mg/day.

Proliferation of Uterine Endometrium in Estrogen-primed Rabbits (Clauberg Mcphail Test)

Female rabbits weighing an average between 800 to 1000 grams were primed with estradiol followed by the administration of progestational compound will then produce rapid growth and multiplication of the endometrium that goes into secretory phase. These requirements are followed during the evaluation of progestational hormone [26, 31, 32, 33]

Procedure: Daily injections of estradiol in an aqueous solution at a concentration of 0.5 mcg/ml are administered to female rabbits weighing from 800 to 1000 grams, The dosage is increased on the seventh day of drug treatment The complete dose is delivered in five equally divided fractions, and each fraction is administered daily over 5 days. After the last injection, animals are euthanized and an additional 24hr time is allowed. After that, the uteri were removed, frozen sections were made and analyzed for histological interpretation of a certain part from one horn. Growth of the endometrium, prior to pregnancy. The grade 4 referred to the onset of glandular development [34].

Measurement of Carbonic Anhydrase Activity in the Endometrium of Rabbits

In the endometrium, we are likely to see a direct correlation between the rabbit administration of Progestogens and the activity of carbonic anhydrase. This procedure is used for the amplification of progestational chemical [26, 35].

Procedure: The immature female rabbit was used in this study via priming their sexual maturity by estrogen before the injection of both the test medication and standard drug. Euthanize animals after conducting the drug treatment study, then remove the uteri. Calorimetry to assess the carbonic anhydrase activity of the endometrial extract provided from uterus [35].

Deciduoma Response in Rats

This study focuses on the principle of maternal placental tumor growth caused by progestational damage in the uterus or removal of the ovaries. This phenomenon is utilized for the evaluation of progestational chemicals [36].

Procedure: The present study was performed on atomized adult female albino rats of 150 to 200 grams weight. That rat was then prepared by giving four injections, each of 1 mg estrone/estradiol. The second step is a 9-day cycle of pharmacological treatment. The open uterine horn treated with one milligram of histamine dihydrochloride is injected into the inner cavity per day for five days. The animals are then euthanized at 24 hours after the final dose of medicine. preparation of the surgically removed uterine horns as preservation in paraffin, followed by histological analysis [37].

In Vitro Methods

Strategy followed for progesterone receptor binding assay It is predicated on the principle of competitive binding where within the assistance and unlabelled progesterone molecules bind to the progesterone receptors. Progesterone compounds displace labelled progesterone from the progesterone receptor, in a concentration dependent competitive manner [38, 39].

Procedure: The human Female uterus retrieved after hysterectomy has been cryopreserved in liquid nitrogen at -80°C until utilization. Cytosol was prepared the process of mincing uterine tissues followed by homogenizing them with an amount using a homogenizer at 0 to 4°C. In which is ice-cold buffer consists of 10 mM KH₂PO₄, (M K₂ HPO₄), (M EDTA), NaN₃ and 10% glycerol pH7.5 refers to as PENG Buffer The homogenates are then centrifuged at a temperature of 4 °C for 30 minutes at a speed set to be equal to or more than 10,500 g. The liquid is then extracted and is known as cytosol

Anti-progestational Activity

The anti-progestational compound inhibits certain or all of the biological impacts of progesterone. This principle is employed to evaluate the anti-progestational activity [26]. The Clauberg/McPhail assay protocol for deciduoma development is modified by administering the test drug in conjunction with progesterone.

Anti-Progestational Activity in Immature Rabbits (Mc Ginty Test)

Procedure: Anti-progestational activity was determined as previously described by McGinty et al. in 1939 [40]. Immature rabbits prior to the experiment received estrogen for 6 consecutive days, with an exception of control animals. Positive control group: The right uterine horn was injected with 0.1 mL of progesterone, and the left horn received an injection of 0.1 mL. A solution that consisted in the association of progesterone and test drugs (Ranitidine, Famotidine, Roxatidine) diluted in 0.5% carboxymethyl cellulose was applied into the left uterine horn; meanwhile only progesterone was administered into the right horn of uterus [3]. Rabbits were sacrificed three days post-inoculation and uteri were collected, weighed and fixed in 10% buffered neutral formalin. Recordings were assessed histopathologically according to McPhail scores [41, 42]. Statistical analysis was performed on the mean uterine weights using Student's t-test.

Anti-implantation activity oral route [44]

A total of between 180 and 200 grams of female Wistar rats were mated with adult male rats at a ratio of three females to one male. Phenomenon indicating the beginning of pregnancy day zero can be defined as the initial day of vaginal smears after intercourse that is positive for spermatozoa. Pregnant rats were divided into appropriate groups, including an experimental and a control group. The experimental group was given H₂ receptor blockers orally on days: 1–7 and day 4 of pregnancy, each at three doses. To assess the impacts, on the 10th day a laparotomy was performed and corpora lutea as well as implantation sites were counted. Afterwards, these rats were allowed to reproduce and their progenies which were produced by them were counted. The study calculated the percentage of antifertility action,

pre-implantation loss and post-implantation loss in investigated parameters [45]. Statistical analysis was performed using Student's t-test [43, 44].

Anti-implantation activity (Bilateral intra-uterine administration)

“This can be seen from the same algorithm as described by Upadhyay et al. (2014)” Intrauterine injections were performed as previously described. This technique consisted of opening the abdominal cavity in the midline and directly administering diverse compounds into each uterine horn close to the uterus-cervix junction (approximately 0.5 cm). The experimental animals from the test groups were administered with ranitidine doses of 7.0 and 14 mg/0.1 ml/u.h, famotidine doses of 1.0 and 2 mg/0.1 ml/u.h, roxatidine doses of 3.5 and 7.0 mg/0.1 ml/u.h. For ranitidine and roxatidine control groups, 0.1 ml of sterile saline; for famotidine, the control group was treated with 0.1 ml of diluted saline in a bulk gum acacia solution (concentration: 1%). To inhibit the response, after the injection, forceps were clamped on either side of the uterine horn for 30 seconds as shown in Figure (3). After the injection was administered, the abdominal wall was stitched together and skin closed back in place, with rats returning to their cages.

On the 10th day, another laparotomy was performed to determine corpora lutea and implantation sites count in each group, inspection for embryo/pups alive excluding resorbing fetus. Healthy embryos were characterized by bright reddish colour with clear margins, whereas resorbing fetuses were dull blue in appearance with no visible margins or orientation and frequently surrounded by exudate (sensu Batta and Martini 1975) [45]. Later, the rats were allowed to mate and the number of offspring produced was recorded. “Antifertility activity was evaluated by the rates of pre-and post-implantation losses.”. Statistical Analysis: Statistical analysis was performed on the result using a one-way ANOVA test.

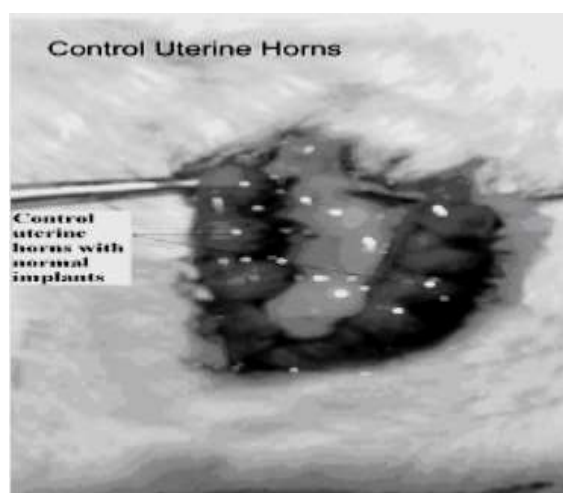


Fig.3 After receiving a bilateral intrauterine injection of sterile saline, control the uterine horns. The implants that developed adhered to standard orientation and spacing [44].

Abortifacient Activity

Procedure: The study used adult female albino rabbits. The gestational period starts from the date of mating observation After 12 days of gestation palpation method can be used to

confirm the pregnancy. Intra-amniotic and intra-placental injections are given exactly on day 20 of rabbit pregnancy; by using ether, the rabbit gets introuvable anesthesia. The antrum of the uterus was entered through the midline incision, a spot for injection was selected and its various components were identified by transillumination using a powerful light source zero.1 ml of solvent is injected into the amniotic fluid or zero.05 ml of solvent administration within the placenta. Treatments can also be performed or the substances provided using any route of administration and from day 20, consecutively for at least one animal in each group. The effect of the drug is evaluated on these parameters like vaginal bleeding, weight changes and abdominal palpation as well as postmortem examination [47].

Methods for Males

The development of male antifertility medicines entails disrupting spermatogenesis while preserving desire and secondary sexual traits.

Emergent Spermatozoa Made Nonfunctional/Oligospermia/Aspermia

In vivo Methods

Cohabitation Test

This experiment measures the duration between the introduction of treated males to two females and the development of litter. The date of mating is determined based on the date of giving birth. This approach is appropriate for medications that are recognized to induce a period of infertility lasting multiple weeks [48].

The research involved adults of both genders of albino rats with a pronounced fertile state. Animals were kept at a 2:1 male-to-female ratio for breeding until both females had litters. The day of the meeting depends on the date of the parturition. The time between the first introduction of treated meals into females and the date before when no/minimal production was observed is measured [21, 48].

In Vitro Methods

Spermicidal Activity

Procedure: Spermicidal drugs are incubated with normal saline and serially diluted in 0.2 ml using 1 ml spermicide solution into human seminal fluid After this, the mix is kept in an incubator whose temperature was set at 37°C for half an hour. A drop from the mixture was immediately put on a slide and 5(+) fields (high-power) were examined for changes in shape and movement demonstrated by the sperm. Cells that have no discolouration are alive ones. Active agents can immobilize the spermatozooids and bring about their death [49].

Immobilization Assay

Procedure: The segmented cauda segment of the epididymis of the ram is isolated and minced finely in 0.9% saline containing approximately a pH of 7.5. This mixture passes through a coffee filter to obtain sperm, (a sperm suspension). human, ejaculates (n = 10) were

collected from healthy proven individuals who had abstained from sexual activity for <72 to 96 hours. The resultant ejaculates are then analyzed by standard semen analysis procedures and liquefied at 37°C. A sperm concentration of about 100 million/ml, a motility percentage above 60% with normal morphology, as well as rapid progression, is necessary for the test to be conducted [24]. The sperm suspension in 100 million/ml to 200 million/ml (epididymis of rats) or human ejaculate with the concentration between 100 and 150 ($\times 10^6$ cells/ml. d) pills are then mixed consistently at a ratio of 1:1 from various dosages. A drop of the mixture was immediately put onto a slide and at least 5 different fields were observed under high power (400 $\times$) in each preparation using a microscope to observe the motion of sperm. Finally, the combination is incubated for 30 minutes at a temperature of 37°C before repeating these steps [49, 50].

Spermicidal Action of Hydro-methanol Extract from *Tinospora Cordifolia* (Willd)

The study conducted by *Puja Das et. al*, [51] examined the role of human fertility regulation in controlling over-exploitation and its associated environmental effects. The present study aimed to assess the in vitro effect of hydro-methanol extract obtained from *Tinospora cordifolia* stems on both human as well as rat samples to kill sperm and reproductive dysfunctions. “In our experimental performed in the setting, five groups were evaluated: control and treated with 0.5, 1 and 2mg of TCHME; this parameter comparison permitted us to verify the effects exerted by melatonin”, said the study. At dosages and durations of exposure relative to the control group, it was observed that TCHME significantly ($p < 0.05$) reduced sperm motility, viability, plasma & acrosomal membrane intactness as well as nuclear chromatin de-condensation indicating a decrease in spermiological parameter values as depicted in Table 2. The IC₅₀ values of TCHME on human and rat sperms were 0.8, and 0.4 (mg/ml) for motile gender respectively [51].

In sperm pellets, testes and epididymis of rats administered with TCHME showed distinct decreases any exerted ($p < 0.05$) in the levels of critical androgenic enzymes important to testosterone synthesis; and antioxidant enzymes (geneticone based on these findings on model-generated parameters). Conversely, although the activity of antioxidant enzymes in renal tissue significantly ($p < 0.05$), it was not statistically significant ($p > 0.05$) for hepatic tissues, which also increased. The study recorded a significant increase $p < 0.05$ in the levels of thiobarbituric acid reactive substances TBARS in the sperm pellets, testes, and epididymis of group I respectively. However, in TCHME-treated groups, the TBARS levels were significantly ($p < 0.05$) decreased compared to CCl₄-induced rats’ liver and kidney tissues for all dose levels of rats. However, since the levels of GOT and GPT had no significant alterations in these organs ($p > 0.05$), TCHME may have no or minimal harmful effect. These results in sum show that TCHME exert a more pronounced effect on inducing stress not only in the reproductive tissues but also in sperm as compared to other organs. This study has for the first time demonstrated TCHME spermicidal and fertility properties, with the ability to target specifically reproductive tissues which might be useful in developing a prospective male contraceptive utilizing this plant as raw material [51].

Conclusion

Antifertility agents in animal models as a recent development bring together various contraceptive practices and mechanisms where not only the female but also the male reproductive system is involved. The study discusses various methodologies and assays used to evaluate the efficacy and safety of candidate antifertility agents. In addition, female contraception could be provided by anti-ovulatory activity evaluation of oral HCG and rats-induced ovulation mode versus rabbits' cupric acetate -interventional induction modalities; they all offer precious in-vivo practical assistance to elucidated mechanisms of fertility control. A combination of in vivo and in vitro methods for the assessment of estrogenic activity, such as the estrogen-receptor-binding assay employed here, provides additional information on hormonal pathways contributing to reproductive physiology.

Similarly, progestational activity and pregnancy maintenance assays help to clarify the mechanisms of action for progestin-based contraceptives, necessary for the development of reliable and safe contraceptive alternatives for women. Taken together, these in vivo and in vitro approaches could enable researchers to determine the possibility of antifertility agents for avoiding pregnancy as well as being lodged pregnancy. In addition, the review discusses how spermicidal activity assessment and immobilization assays can be key to establishing male contraceptive options. This indicates means by which potential agents may act to block sperm motility and other functions in preventing fertilization. To conclude all these advancements the above summarized literature explains that animal models for contraception are an essential requirement which overcomes human causes and helps in the development of new antifertility agents with good efficacy, safety, user compliance etc. Diverse methodologies and animal models can be further leveraged by researchers to help in the advancement of contraceptive practices, which will eventually prove beneficial for efforts directed towards global reproductive health and family planning.

Limitations

Despite the substantial progress made in animal models that have greatly contributed to our insight into optimal contraceptive methods, there are several drawbacks. The diversity in reproductive physiology between different animal species thus limits the extent to which these results may be directly applied or translated as a method of human contraception. Scientific necessity implies that animals are used under the belief of what is best for society or in an attempt to gain exceptional scientific knowledge. Ethical responsibility asserts a government's duty to protect individuals from unnecessary harm because of experimental activities. Thus, ethical issues arise where these two principles do not adhere. In addition, the predictive validity of animal models to humans for response to antifertility agents was also doubtful because of interspecies variation in metabolism and pharmacokinetics⁸⁶⁷. In addition, especially concerning hormone-based contraceptives, animal models may not completely reflect the complex reproductive physiology and hormone regulation that underlie potential contraceptive failures or side effects. Moreover, in animal studies, it is not easy to undertake a complete evaluation of the long-term and reversibility of fertility after the withdrawal of antifertility agents. In the end, though animal models do provide some

incredibly useful advantages when conducting contraceptive research, overcoming these challenges will often require sustained interdisciplinary collaboration and adherence to responsible research practices.

Author Contributions

JT, writing original draft

MIG, Review

SH, Data correction and language improvement

GMI, Review

SKD, Review and proofread

AA, Writing

YS, Writing

AKG, Data gathering

M, writing draft

SS, Review

SK, Review

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