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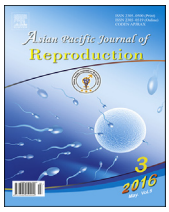

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An overview of the current methodologies used for evaluation of anti-fertility agents

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ABSTRACT

Discoveries in the past two decades have continued to improve our understanding of the mechanism of fertilization and animal models have played a significant role to define the basic mechanism of anti-fertility agents. *In vivo* models have been developed in the past years to study the anti-fertility agents. Methods that are used in anti-fertility study can be categorized into method including estimation of sex hormones, assessment of sperm motility and count, assessment of sperm viability and morphology, mating trial test body, sex organ weights, abortifacient activity, post-coital anti-fertility activity, effect on estrous cycle, anti-estrogenic activity, anti-gonadotrophic effect and quantification of fructose in seminal vesicle, histopathology, and biochemical methods. This review aims to highlight some of the new and currently, used experimental models that are used for the evaluation of anti-fertility agents.

1. Introduction

The population explosion is a leading cause of poverty and pollution in developing countries [1]. Exponentially growing population has been adversely affecting the social, economical and technological development of human race [2]. Therefore to reduce/control our number has to be the first on a priority list. A good number of synthetic contraceptives are available in market, each one with either a limited success or side effects [2]. It created a population control programme, which includes studies of traditional medical practices [1]. Since ancient times, plants have been a source of drugs, but scientific medicines tend to ignore the importance of herbal medicine [3]. The World Health Organization suggested that effective, locally available plants can be used as substitutes for drugs [1].

Medicinal plants in India have been screened for contraceptive potential and anti-fertility effects, since the country has always been concerned about population explosion [1]. Exploration of drugs having anti-fertility activity is the need of current time, and many time plant extracts have been investigated for their anti-fertility effect in animals [4]. Since herbal drugs are easily available and with no side effects, the current study was undertaken [2].

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2. 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.

2.1. Parameters used to evaluate anti-fertility agents

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

2.2. 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) [5].

2.3. Assessment of sperm motility and count

Progressive motility was tested immediately. The right cauda epididymis 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 ×40. The total numbers of sperm cells were counted and expressed as 10^6/mL [5].

2.4. 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 ×400 magnification. Viable sperm remained colorless while non-viable sperm stained red. The stained and the unstained sperm cells were counted using ×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× magnifications. A total of 300 sperm cells was examined on each slide (1 500 cells for each rat), and the head, tail and total abnormality rates of spermatozoa were expressed as a percent [5].

2.5. 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:

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

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

Fertility index = number pregnant/number mated × 100 [5].

2.6. 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/body weight × 100) [5].

2.7. 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 μL of zinc sulfate and sodium hydroxide to make a total dilution of seminal plasma 1:16, followed by centrifugation at 2 500 r/min for 15 min. For fructose measurement, 200 μL 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 μmol/mL of seminal plasma [5].

2.8. Abortifacient activity (Anti-implantation activity)

The plant extracts were tested in female albino rats for abortifacient activity. The vaginal smears of caged female rats of known 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 proven fertility in the ratio of 2:1, in the evening of proestrous and examined the following day for the evidence of copulation. Rats exhibiting thick clumps of spermatozoa in their vaginal smears were separated and that day was designated as day one of pregnancy. These rats were randomly distributed into four groups, a control group and three experimental groups of six animals each. Group I received vehicle only and served as control. Groups II, III, and IV received different extracts. On the 10th day of pregnancy the animals were laparotomized under 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, specified by an intragastric soft rubber catheter from day 11 up to the 15th day of pregnancy. The animals were allowed to go to full term. After delivery the pups were counted and the antifertility activity of extract was evaluated. Litters were examined for any malformation [1,6,7].

Percentage abortifacient activity = number of resorptions/number of corpus luteum × 100 [8].

2.9. 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 luteagraviditis [9–12].

Frequency of pre-implantation losses = missing no. of implants (corpora lutea implants)/no.of corpora lutea × 100