Evaluation of the effect of *Ficus umbellata* extracts on estrogen and cytological variations in the vaginal epithelium in *Oryctolagus cuniculus*

ABSTRACT

Aims : To evaluate the effect of aqueous extracts of *Ficus umbellata* on the stimulation and ovulation in rabbits.

Study design: Experimental design

Place and duration of the study: Laboratory of Histology, Embryology and Cytogenetics, Biochemistry Laboratory of the Medical Sciences, Unit of Medical Sciences of Abidjan and Laboratory of Biological Sciences, Unit of Pharmaceutical and Biological Sciences, Felix Houphouet-Boigny University, between January to June 2025.

Materials and methods: The aqueous extract of *Ficus umbellata* was obtained from fresh figs that were crushed and then dried to produce a powder. The animals consisted of 12 weaned New Zealand rabbits weighing between 1,8 and 2,5 kg. They were treated according to the Helsinki 2024 protocol. They were divided into four groups of three animals each. Groups III and IV were treated with *Ficus umbellata* at doses of 150 and 300 mg/kg/day of body weight, respectively. Group II was treated with clomiphene citrate (50 mg/kg/day). The animals were force-fed using a rat cannula adapted to a section of IV tubing. Group I received distilled water. Analyses were performed, such as blood estradiol testing, vaginal smears to determine estrogen index (IO), and histological sections of the vagina.

Results: Administration of the treatment concentrations did not show any changes in histomorphological parameters. A weight gain of 2,06 ± 1,6 kg has been observed with animals treated with 300 mg/kg/day.

A non-significant increase in blood oestrogen concentrations. However, the treated groups had retained OIs above 60% (group II=63,3%, group III=83%, group IV=77,3%). Then, estrus has been induced in the groups of rabbits that received clomiphene citrate and *Ficus umbellata*.
Conclusion: These results confirm that the aqueous extract of *Ficus umbellata* figs has follicle-stimulating activity.
Keywords: *Ficus umbellata*, histomorphological, follicle stimulation, vagina, estrogen, *Oryctolagus cuniculus*

INTRODUCTION

In sub-Saharan Africa, infertility is a serious issue, with rates ranging from 15 to 30% (Rambert, 2016). It is primarily a social problem rather than a therapeutic one, due to the stigma attached to childless couples. Specialized infertility care is provided by assisted reproductive technology (ART) centers. Various treatments are available, including clomiphene citrate, LHRH pumps, surgery, in vitro fertilization (IVF), intrauterine insemination (IUI) and ovarian stimulation (Azonbakin et al., 2022). In developed countries, the use of ovulation inducers to stimulate the ovaries has transformed the prognosis for infertility and contributed to assisted reproductive technology (Faure, 2015). Clomiphene citrate is a competitive inhibitor of estradiol (E2) at its hypothalamic receptors and is likely to cause an increase in serum E2 levels (Mazzola et al., 2019).

Although assisted reproductive technologies have been around for over 30 years, they remain unaffordable and inaccessible in many parts of the world, particularly in low-income countries (WHO, 2020).

In Africa, few couples are aware of the existence of these specialized centers, and most consider them to be beyond their means for financial reasons. Forty per cent of couples do not visit a health facility but seek help from people with no medical training. The rest go to primary care centers (Rambert, 2016). As a result, the use of traditional medicines to induce folliculogenesis and spermatogenesis could be an alternative for effective and less costly treatment of infertility. In Africa, several studies have been conducted in this area. For example, the study by Koman et al. (2019) identified and documented the medicinal plants traditionally used by traditional birth attendants to treat female infertility in the department of Dabakala (Côte d'Ivoire). The results of this study indicated that 25 species from 23 genera and 18 families are used to treat various cases of infertility. The most represented families are *Annonaceae* and *Solanaceae*. Among the species cited, *Heliotropium indicum* (*Boraginaceae*), *Parkia biglobosa* (*Fabaceae*), *Xylopia aethiopica* (*Annonaceae*), *Vitellaria paradoxa* (*Sapotaceae*) and *Kigelia africana* (*Bignoniaceae*) are the most recommended. Moyabi et al. (2021) showed that 65 species, including *Ficus umbellata* (*Moraceae*), are used in treatment of human infertility. Similarly, Coulibaly et al. (2023) evaluated follicle-stimulating activity in the female rats treated with Cissus aralioides extract. The results of administering different concentrations of the aqueous extract showed no toxicity in the treated rats. Furthermore, the determination of the estrogenic index revealed follicle-stimulating effects. It appears that several plant species are used in the treatment of infertility. Among these plants, the genus *Ficus umbellata* (*FU*) has been the subject of numerous studies. However, very few studies have highlighted its action on the reproductive system.

This work was undertaken with the aim of determining the stimulating effects of aqueous extracts of *Ficus umbellata* figs in female rabbits. This animal was chosen in accordance with the work of Junqueira et al. (2018), which showed a similarity with the vaginal epithelium of women.

The overall objective of this study is to evaluate the follicle-stimulating activity of *Ficus umbullata* on the vaginal epithelium of female rabbits. The specific objectives are:

- To determine morphometric parameters of rabbits during treatment
- To determine the estrogenic indices of rabbits in the experimental groups

- To compare variations in circulating estrogen levels in rabbits from different groups

I. MATERIALS AND METHODS

1.1 Type, period, and location of study

This was a comparative experimental study conducted from January to June 2025, i.e., over a period of six months. The study was carried out at Felix Houphouet-Boigny University in Abidjan. The animal experiments were carried out at the animal facility of the Faculty of Pharmaceutical Sciences, the estrogen dosage at the Biochemistry Laboratory of the Medical Sciences Research Training Unit in Abidjan, staining of vaginal smear slides was carried out at the Histology, Embryology, Cytogenetics Laboratory, and histological slides were stained at the Pathological Anatomy and Cytology Laboratory of the Faculty of Medical Sciences in Abidjan.

1.2 Animal material

The material consisted of 12 apparently healthy female New Zealand rabbits, weaned at one month of age and weighing between 1,8 and 2,5 kg. They were treated according to the Helsinki 2024 protocol. These animals were acclimatized in individual cages, fed 120 g of pellets per individual and regularly watered to reach a fertility weight of between 2,4 and 6,3 kg. The building was lit by daylight.

1.3 Plant material

The plant material used was figs from *Ficus umbellate*, harvested in the National Floristic Center of Felix Houphouet-Boigny University of Abidjan, and compared to authentic specimens registered. The fresh figs were first crushed and then dried at room temperature for two weeks to produce a powder. Next, 100 g of powder was macerated in one liter of distilled water by blending in a mixer. The solution was then filtered through cotton wool. The filtrate obtained was evaporated to produce an aqueous extract. The macerate obtained was filtered twice through cotton wool and once through 3 mm Whatman filter paper. The filtrate obtained was concentrated using a Büchi rotary evaporator at 60°C. The paste was collected and freeze-dried. The extract thus obtained is the total aqueous extract.

1.4 Experimentation

The female rabbits were divided into four groups of three animals each. The animals were force-fed using a rat cannula adapted to a section of infuser tubing. Group I received distilled water (negative control).
Group II, treated with clomiphene citrate, received 50 mg per day in a
5 mg/ml solution, also respecting the standardized volume.
Group III treated with *Ficus umbellate*, 150 mg/kg/day
Group IV treated with a dose of 300 mg/kg/day of body weight.

1.4.1 Performing vaginal smears

The vaginal smear consisted of spreading a sample of vaginal cells onto a numbered glass slide using a single-use cotton swab moistened with distilled water. After fixing with strong lacquer and drying in the open air, the smears were transported to the laboratory for staining. Three stains were used (Papanicolaou stains): Harris hematoxylin, orange G, eosin azure and light green SF at different stages. Harris hematoxylin stains cell nuclei due to its affinity with DNA. Orange G reacts with immature squamous cells (basal and intermediate cells). Eosin azure and light green SF stain the cytoplasm of metabolically active cells blue. The smears were read under a 40x magnification optical microscope to determine the percentages of basal cells, intermediate cells, and superficial cells.

1.4.2 Measurement of circulating estrogen levels

Weekly blood samples were taken using a 5cc syringe from the marginal vein (ear) of each rabbit. The whole blood samples were collected in red-capped tubes. After coagulation, the coagulum obtained was centrifuged. The serum was then collected in an Eppendorf tube for storage in the refrigerator. The analysis method was based on fluorescence immunoassay technology using the WONDO Finecare PLUS FIA Meter II Plus SE device. The sera contained in the dry tubes were mixed and placed in the well of a cassette, which was then inserted into the device. This device uses a fluorescence immunoassay technique, specifically the sandwich immunoassay method. The analysis was performed by mixing the antigen and antibody, followed by the formation of a complex on a nitrocellulose layer. Finally, the results were read and interpreted, with the intensity of the detector antibody signal reflecting the amount of antigen and the Finecare counters indicating the estrogen concentrations in the sample.

1.4.3 Histological section of the vagina

To perform the histological sections, the animals were anaesthetized with ketamine at a dose of 0.1 mg/kg body weight, sacrificed and dissected in the Histology, Embryology and Cytogenetics Laboratory of the Faculty of Medical Sciences at Felix Houphouet-Boigny University.
The vaginas were removed from 2/3 of the organ at the end of the study and fixed in 10% formalin. They were placed in identified cassettes for the dehydration stage (passage through different alcohol baths of increasing strength). The cassettes were placed in toluene baths (clarification), then impregnated in molten paraffin and embedded in molds to make blocks for histological sections. The microtome was used to obtain thin sections (2 to 5 µm thick). Hematoxylin/eosin staining and mounting are carried out after spreading, uncoating and rehydration.
Hematoxylin/eosin stains the cytoplasm pink, the nucleus purple and the collagen fiber orange-yellow.

1.4.4 Variables studied

The variables in this study included quantitative variables such as weights 1 and 2, representing the weights at the start of treatment and at the end of treatment, respectively. The morphometric determinants of the vagina (weight, length and width) were also studied, as well as the circulating estrogen level.
The qualitative variable was the estrogen index (OI), defined as the percentage of superficial eosinophilic cells. This is a cytological marker used to estimate estrogen impregnation and is determined by the proportion of superficial cells in a population of 100 cells.

II. RESULTS

2.1 Anatomy of the female rabbit's vagina

The female rabbit's reproductive system measures approximately 20 cm in length and consists of, from the outside to the inside, a vagina (A) (7,1 ± 0,5 cm in length), uterus (B) (4 cm ± 0,1 cm in length), uterine horns (C) (8± 0,5 cm in length) and ovaries (D) (2 cm in length). The bladder and urethra are contiguous on the posterior side at the level of the vagina (Figure 1).

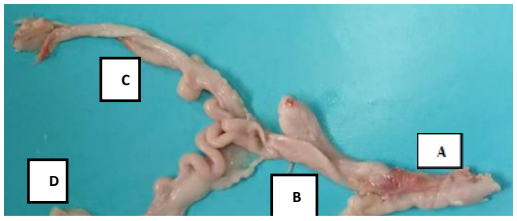

Fig.1: Anterior view of the female rabbit's genital apparatus

A : Vagina, B: Uterus, C: Uterine horn, D: Ovary

2.2 Variations of morphological parameters

The different averages of rabbit's weights have been recorded in the table 1. At the beginning of the experiment, the groups of animals had homogeneous weights with a difference not exceeding 100 g. However, at the end of the experiment on day 30, the animals in group IV had doubled their weight compared to the weights of the animals in the other groups. They are following by group III animals. The lowest weight gain had been obtained in group II treated by the reference molecular of clomiphene.

Table I: Distribution of average weights of female rabbits according to treatment

LOTS	WEIGHT (Day=0)	WEIGHT (Day=30)	WEIGHT GAIN
Group I	2233,3 ± 251,6	3233,3 ± 152,7	1000 ± 98,9
Group II	1900 ± 251	2666,7 ± 152,7	766,7 ± 98,3
Group III	2533,3± 208,2	3433,3± 378,5	900 ± 170,3
Group IV	2033,3± 251,6	4100± 1905,2	2066,7 ± 1643,6

This table shows an increase in the weight of the does in the different batches. These differences are not significant (p=0,3). The does in batch 4 have a higher average weight gain than the does in the other batches.

2.3 Estrogenic index (IO)

Observation of vaginal smears before, during and after treatment revealed various cells such as epithelial cells (superficial, intermediate, parabasal, basal), blood cells such as polynuclear cells and red blood cells (Figure 2).

Vaginal smears taken at the start of treatment showed a high IO value in group II, at 71,7 ± 11%. This was compared to group III (68,3 ± 14), group IV (60,3 ± 25%) and group I (69,7 ± 18). However, the comparative analysis of variances did not show a significant difference (p=0,9). During treatment, the highest IO value was 87 ± 3,6% observed in group IV. Groups I, II and III had average IO values of 67,7 ± 19%, 66 ± 24% and 85 ± 8%, respectively.

At the end of treatment, the rabbits in the treated groups had retained OIs above 60% (group II=63,3%, group III=83%, group IV=77,3%). The rabbits in the groups that received clomiphene citrate and *Ficus umbellata* were in estrus.

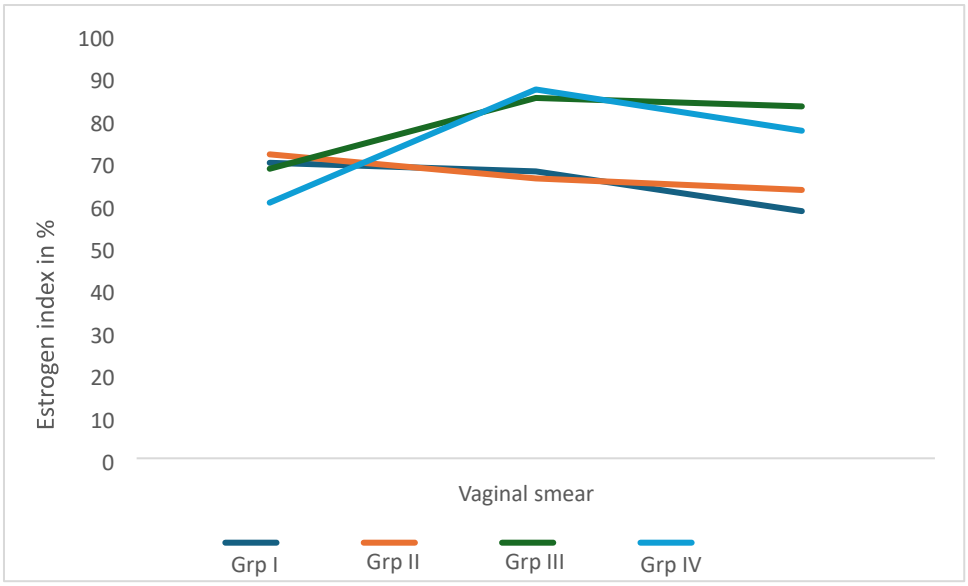

Figure 2: Evolution of estrogenic index in female rabbits according to treatment

2.4 Variations in the histological structure of the vagina of female rabbits

Observation of the various histological sections showed very little variation in the structure of the vagina. In the vaginal mucosa of all female rabbits, the epithelium consisted of a layer of cylindrical (glandular) cells. The nucleus of these cells was located at the basal pole, and the apical pole was occupied by mucus (Figure 3).

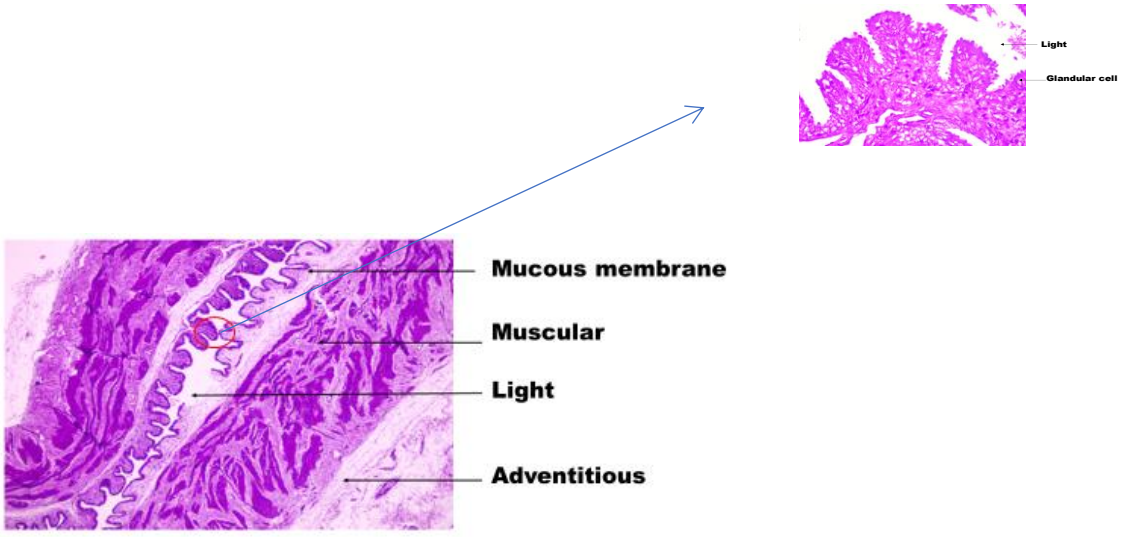

Fig.3: histological section of the vaginal wall of a rabbit, x 400, Hematoxylin-Eosin

2.5 Estradiol levels

Blood tests for estradiol before and after treatment showed different variations in concentration between treated and untreated rabbits (Table II). An increase in average estrogen concentrations was observed in all Groups. The highest value of estrogen was observed in Group IV (776,4 ± 184 pg/ml). The control group I had the lowest value about 179 ± 90 pg/ml. The average estradiol concentrations in the control rabbits were not significant (p=0,39) compared to those in the treated rabbits.

Table II: estradiol levels according to treatment

LOTS	**Rate 1** (pg/ml)	**Rate 2** (pg/ml)	**Rate 2- Rate 1**
Group I	179 ± 90	188,7 ± 56	29,4 ± 31
Group II	203,2 ± 63,2	208,4 ± 59	14,5 ± 7,2
Group III	234,4 ± 134	257,2 ± 129	22,8 ± 5
Group IV	713,7 ± 187	776,4± 184	62,7 ± 3

DISCUSSION

The study has shown a gain in weight variations in rabbits treated with *Ficus umbellata*. A variable weight gain between 766 and 2066 g was observed in female rabbits that received the aqueous extract of *Ficus umbellate* at different dose. This result differed from Oka (2023) who did not find a weight gain within *Ficus umbellata* administration. This situation could be explained by the fact that ficus figs are very energetic (Okon et al., 2025), and OKa's experiment only lasted a week, a short time to appreciate the effect of the figs.

The average vagina length was 7,1 ± 0,5 cm. This value was consistent with Salissard (2013). In general, the average length of the vagina in female rabbits is between 4 and 8 cm, with a width of 1 to 1,2 cm. Furthermore, the wall of the vagina is thin, giving it a flattened shape.

Blood tests for estradiol has shown different variations in concentration between treated and untreated rabbits. An increase in average estrogen concentrations was observed in all groups while the mean estradiol concentration in the control rabbits were not significantly different from those in the treated rabbits. Moyabi et al. (2021) demonstrated the estrogenic properties of aqueous extracts of *Ficus umbellata*. Silihe (2021) considered *Ficus umbellata* to be a plant used in Cameroonian pharmacopoeia for the treatment of certain physiological disorders associated with menopause. Studies have revealed that aqueous and methanolic extracts of its bark exhibit estrogenic and anti-estrogenic activities. Zingue et al. (2016) isolated the major compound of *Ficus umbellata*, 7-methoxycoumarin (MC), and evaluated its estrogenic effects in vitro and in vivo. The results showed that the MC compound exhibits weak estrogenic activity, both in vitro and in vivo, indicating that the estrogenic effects of the total plant extract are mainly attributable to other phytoestrogens. Nevertheless, MC could be a relevant therapeutic option for the management of vaginal dryness, due to its targeted activity in vaginal tissues, without inducing proliferative stimulation in the endometrium, thus limiting the risk of uterine carcinogenesis. Observation of vaginal smears has revealed various cells, such as the epithelial cells listed below, from the lumen to the basement membrane. Superficial cells are the outermost layers, flattened and polyhedral with acidophilic cytoplasm (bright pink/orange) and small or absent nuclei (pyknosis). Parabasal and

intermediate cells have round, dense nuclei with basophilic cytoplasm (light blue/green). Basal cells originate from the deep layer of the basal epithelium and are small cells with large nuclei and basophilic cytoplasm. Blood cells consist of polynuclear cells and red blood cells. Vaginal smears taken at the start of treatment of the rabbits showed a high IO value in batch 2 with 71,7 ± 11%. This was comparable to batch 3 (68,3 ± 14), batch 4 (60,3 ± 25%) and batch 1 (69,7 ± 18). However, the comparative analysis of variances did not show a significant difference (p=0,9). During treatment, the highest IO value was 87 ± 3,6% observed in batch 4. Batches 1, 2 and 3 had average IO values of 67,7 ± 19%, 66 ± 24% and 85 ± 8%, respectively. At the end of treatment, the rabbits in the treated groups had retained IO values above 60% (group 2=63,3%, group 3=83%, group 4=77,3%). The rabbits in the groups that received clomiphene citrate and *Ficus umbellata* were in estrus. Estrogen impregnation and estrogen index determination in the vaginal epithelium of the rabbits before and during treatment were high, with an IO>60% of superficial cells. The abundance of superficial cells shows that the aqueous extract of *Ficus umbellata* fig induced strong follicle stimulation activity on the vaginal epithelium of the rabbit. These results were consistent with those found by Oka (2023), whose mean values ranged from 50 and 68% in rabbits treated with aqueous extracts of *Ficus umbellata* figs, and Coulibaly et al. (2023) found values between 60% and 86,67% in rats treated with aqueous extracts of Cissus aralioides. This activity of the extract is not related to the dose, as observed in rabbits in group III, which were treated with a dose of 150 mg/kg and had a higher estrogenic index than those treated with a dose of 300 mg/kg. These results differ from those found by Oka (2023), who observed high estrogenic index in rabbits treated with high doses (600 mg/kg). Estrogenic index above 60% during this experiment indicate a state of estrus that was almost permanent in females from all different batches. This can be explained by the fact that female rabbits do not have an estrus cycle and ovulation is triggered by an external stimulus. The more or less permanent state of estrus is due to successive and overlapping waves of developing follicles, which induce a certain number of mature follicles on the surface of both ovaries (Khelil 2020). Histological analysis of the sections showed very little variation in the structure of the vaginal wall. The vaginal mucosa of all rabbits consisted of a single-layered cylindrical epithelium. This is a glandular epithelium with mucous secretion. It corresponds to the epithelium of the upper two-thirds of the vagina, as observed by Barberini (1991).

However, according to the author, the lower third of the vagina is covered by a stratified squamous epithelium. It is composed of several layers: basal cells, parabasal/intermediate cells and superficial cells. The epithelium is strongly influenced by the estrous cycle. During estrus, thickening of the epithelium is observed. These different epithelia respond to a chorion made of loose, richly vascularized connective tissue, which also contains fibroblasts, collagen fibers and some immune cells. The muscular layer consists of two layers of smooth muscle, the inner circular layer and the outer longitudinal layer. These layers ensure vaginal contractions during mating and parturition. The adventitia contains fibrous connective tissue rich in blood vessels, lymphatic vessels, nerves, collagen and elastic fibers.

CONCLUSION

This experimental comparative study aimed to determine the stimulating effects of aqueous extracts of *Ficus umbullata* figs in female rabbits. The results of administering the treatment concentrations did not show any change in histomorphology parameters. After observing the estrogenic index, follicle maturation activity was noted. In the future, it would be interesting to consider a larger sample size to better assess the stimulating effects on the reproductive system. Ultimately, clinical trials should be conducted on patients facing infertility issues.

Ethical approval
All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee”

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