

Experimental Infection Effect With *C. Albicans* In Female Rats Uterus

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Abstract

The present study aimed to define the effect of experimental infection with albicans yeast on the histological structure of the uterus of pregnant female albino rats, in which 50 vaginal swaps of pregnant women were obtained using sterile cotton swaps from the women who attended the gynecological examination. Consultation was in hospital and some clinics in the city, and isolates were diagnosed using CHRO Magar media and the Vitek2 Compact system. Then, experimental infection was induced in the reproductive tract of female rats that were injected with a suspension of *C. albicans* yeast at a concentration of 1.5×10^8 cells/ml, once daily until the onset of symptoms. The results of microscopic examination of uterine tissues showed necrosis, partial detachment of the simple columnar epithelium and its desquamation in the uterine cavity, as well as infiltration of inflammatory cells and cell necrosis of the specific page and thickening of the nuclei of several cells, rupture of smooth muscle fibers in the circular muscle layer, irregular arrangement in the longitudinal muscle layer, Necrosis of the lining of the uterine glands, some of them expand into the lumen of the gland, dilation and congestion of a number of fibrous blood vessels and edema in some, an abundance of colloidal fibers and endometrial fibrosis.

Key words: Uterus, Candidiasis, *C. albicans*, Vulvovaginal Candidiasis

Introduction

Fungi are eukaryta organisms, as there are approximately 50,000 diagnosed species living in nature, of which (80) kinds of molds and yeasts cause several diseases for humans and animals alike [1]. Fungi can interact with plants, animals, and humans and establish symbiotic, commensal, latent, or pathogenic relationships [2]. Candidiasis recorded actual infections in all tissues of the human body, especially the mucous surface of the mouth and the reproductive system. *C. albicans* is the most prevalent species and is the main cause of candidiasis, followed by *C. guilliermondii*, *C. tropicalis*, *C. parapsilosis*, *C. krusei*, *C. glabrata*, *C. kefyr*, *C. glabrata. pseudotropicalis* [3], the highest percentage of candida was recorded in samples isolated from the vaginal canal with a rate of 33% [4], and *C. albicans* was the most frequent isolate compared to other isolates, and it was also shown that candidiasis is more prevalent in women of different ages Between (18-39) years [5], the incidence of candidiasis and vulvodynia increases in childbearing years compared to women in menopause [6]. *Candida albicans* yeast is considered by being dimorphic, so it grows in its oval or filamentous yeast shape, depending on the environmental conditions of temperature, pH, moisture and components of the nutrient medium, so that the yeast form can grow on acidic solid media containing sugar and nitrogen organic materials, which is a source of Carbon, and at temperatures below 35°C. As for the

filamentous form, it grows on culture media that contain inorganic nitrogen compounds, as well as culture media containing starchy materials such as Potato Dextrose Agar and Corn Meal Agar, with a pH of 6.5 or more [7].

Microscopic examination of *Clostridium albicans* plants grown in the medium of glucose, yeast and peptone extract after a 3-day incubation period at 25°C shows that its cells (spores) are oval or spherical and sometimes elongated (4-6) µm in diameter, single or in pairs or fungi False deer, its colonies are creamy white, smooth and convex, and presents a yeasty smell when grown in aerobic conditions, while it grows in a liquid medium at the bottom of the tube in a precipitation over an incubation period (24-48) hours. Germ tube when cultured in human blood serum or egg albumin for (2-3) hours at a temperature of 37 degrees Celsius, and one of the important and rapid diagnostic characteristics of this yeast is its ability to form large spherical cells. The form is thick-walled, with a diameter of (8-12) µm, terminal or lateral to the site called chlamydial disc spores [8], and these yeasts are also Gram-positive [9], in addition to the possibility of lactophenol staining in blue to observe Chlamydia spores, and fungal hyphae [10].

The ovaries multiply by forming a bud Spore that quickly separates to form new cells, and when this bud does not separate from the mother cell, a pseudo-mycelium will form, which is known as a small lateral filamentous growth characterized by the absence of true septa, and the terminal cell of the filament is shorter or equal to the cell next to it [8].

The uterus is a pear-shaped organ that involves of two parts, an enlarged upper body called the corpus uteri and a lower cylindrical organ called the cervix. , The uterus is supported by ligaments of tiny sets of fibroelastic components [13].

The Uterine Wall Consists of Three Layers:

1-The outer or perimeter of the uterus / consists of a single row of mesothelial cells supported by a thin layer of connective tissue and is continuous on both sides of the uterus with the peritoneum of the broad ligament.

2-The myometrium / a thick layer of smooth muscle fibers that are separated from each other by connective tissue. The thickness of this layer increases during pregnancy due to a large increase in fiber size, an increase in cell number resulting from the division of the cells themselves, or the differentiation of new cells from the mesenchymal tissue cells present in the connective tissue of the uterus. Three layers of muscle can be distinguished, although their edges are somewhat overlapping, which are the internal muscle, which is the bulk of its smooth muscle fibers arranged longitudinally, and a thick layer of middle muscle whose fibers are arranged circularly and diagonally, and it is rich in blood vessels and a thin outer layer whose muscle fibers are arranged longitudinally located directly under the uterine epithelium.

3-The internal or endometrium is adjacent to the muscular layer and is subject to periodic changes depending on the secretory activity of the ovary. This layer consists of simple columnar epithelial tissue, extending from the surface of the uterine glands, which are separated from each other by a connective tissue that represents the specific stroma or page, and most of the

connective tissue cells in it are also fibroblasts. as lymphocytes and leukocytes, located within a fiber structure of connective tissue, mainly reticular fibers [14].

Working Methods

Samples collection

50 vaginal swabs for pregnant women using sterile cotton swabs were obtained from women attending the gynecological consultation in the hospital and some clinics in the city, under the supervision of a specialist doctor, for the period from the beginning of the fourth month until the ninth month / 2020, for pregnant women in the age group (17-49) years old.

Culture of Samples

Vaginal swabs were transferred directly to the laboratory, and cultured on SDA medium containing the antibacterial Chloramphenicol at a concentration of 10 µg/ml of the medium, and the dishes were incubated in the incubator at 37° C for (24-48) hours, and the dishes that did not show growth were neglected [12].

Purification and Preservation of Isolates

The isolates were purified and cultured on PDA medium containing anti-Chloramphenicol and incubated at a temperature of 37° C for 48 hours, then kept in the refrigerator at a temperature of 4° C, taking into account that it is renewed monthly. As for the preservation of isolates for longer periods of time, it was cultured on slant SDA medium in a sterile small glass bottle, and then incubated at 37° C for 48 hours and kept in the refrigerator at 4° C, with renewal every 3 months [13].

Preparation of yeast suspension

The SDA medium was inoculated with *Candida* by streaking method. The plates were incubated at 37°C for a period of (18-24) hours. Then part of the inoculum grown on the medium was transferred by a loop to a small, sterile, hermetically sealed glass vial containing 5 ml of saline solution. Sterile physiological, mixed well, and then the number of cells was controlled to 810 cells/ml of the solution, by measuring the turbidity of the suspension using a turbidity meter and comparison with the standard McFarland turbidity constant solution [14].

Identification Test

The approved traditional diagnostic methods were followed [15], as well as the use of the PhyTek device.

Experimental Design An experimental infection was induced in the reproductive tract of female albino rats, as they were injected with a suspension of *C. albicans* yeast at a concentration of 1.5×10^8 cells/ml on the seventh day of pregnancy, once daily [14], and continued until the appearance of symptoms.

Tissue Sections Preparation of

Ovarian tissue sections were prepared according to the approved standard method [16], according to the following steps: Fixation, Washing, Dehydration, Clearing, Infiltration, Embedding, Staining, Trimming and sectioning.

Results and discussion

The current study included isolating *C. albicans* fungus from the vagina of pregnant women, as 50 samples of vaginal swabs were examined, and the result was considered positive when the number of colonies was more than ten [19]. The laboratory culture results showed a positive result of 30 swabs, i.e. 60% And 20 smears are negative at a rate of 40%, which is in agreement with the study [6] which showed that microscopic examination is not equal with laboratory culture.

Diagnosis Using CHRO Magar Media

According to the instructions of the medium manufacturer and the specific criteria [21] the isolates grew well to the species level and all appeared in light green, indicating that they are *C. albicans* yeast as in Figure (4-6), and this is consistent with what Al-Obaidi obtained [20].



Figure (4-6): *C. albicans* yeast on chromium agar medium, under 37oC, with age for 48 hours

2-Diagnosis of *C. albicans* Yeast Isolates Using Vitek2 Compact System

The results of the diagnosis of the fungal isolates stated a matching rate that ranged between 6 (9-99%) and these results may be attributed to the possession of *C. albicans* yeast for many of its specific characteristics [22].

The results showed the occurrence of some injuries in the uterine tissue, which was agreed upon with Alvarez and his group [23]. The result of microscopic examination showed necrosis, partial detachment and desquamation of the simple columnar epithelium in the uterine cavity, as well as infiltration of inflammatory cells and cell necrosis of the page. Particular, thickening of the nucleus of a series of cells, eruption of smooth muscle fibers in the

circular muscle layer, irregularity of their arrangement in the longitudinal muscle layer, necrosis of the lining of the uterine glands, and some of them surrender in the lumen of the gland, their expansion and congestion of a series of blood vessels and the occurrence of fibrous edema in some, including It is the abundance of colloidal fibers and endometrial fibrosis, as shown in Figures (1), (2), (3), (4).

Our results were in agreement with Alvarez and his group [23] in the severity of tissue lesions caused by the opportunistic *Candida* fungi, such as degeneration, necrosis and infiltration of inflammatory cells, as well as vascular congestion, which was regarded one of the most important changes that occur as a result of inflammation, and this was also agreed with Jabir [24]. In addition, the increase in pressure leads to bleeding, and the infiltration of inflammatory cells in the affected groups leads to the formation of edema [25].

Van and his group [26] specified that the immune response against *Candida* yeast infection initially includes the infiltration of inflammatory cells that have the ability to devour the pathogen, and this is in line with the current study whose results showed infiltration of cells inflammatory disorders in most tissue sections, such as infection and acute damage, are attributed, To tissues targeted by *C. albicans* virulence factors, including dimorphism, adhesion, secretion of enzymes such as aspartate proteins, toxins such as gliotoxins, and formation of capacitance biofilms, which is one of the most important virulence factors, explains the frequency of its isolation compared to other *Candida* species, Which is alike to those mentioned by Mozni and Shoibki [27].

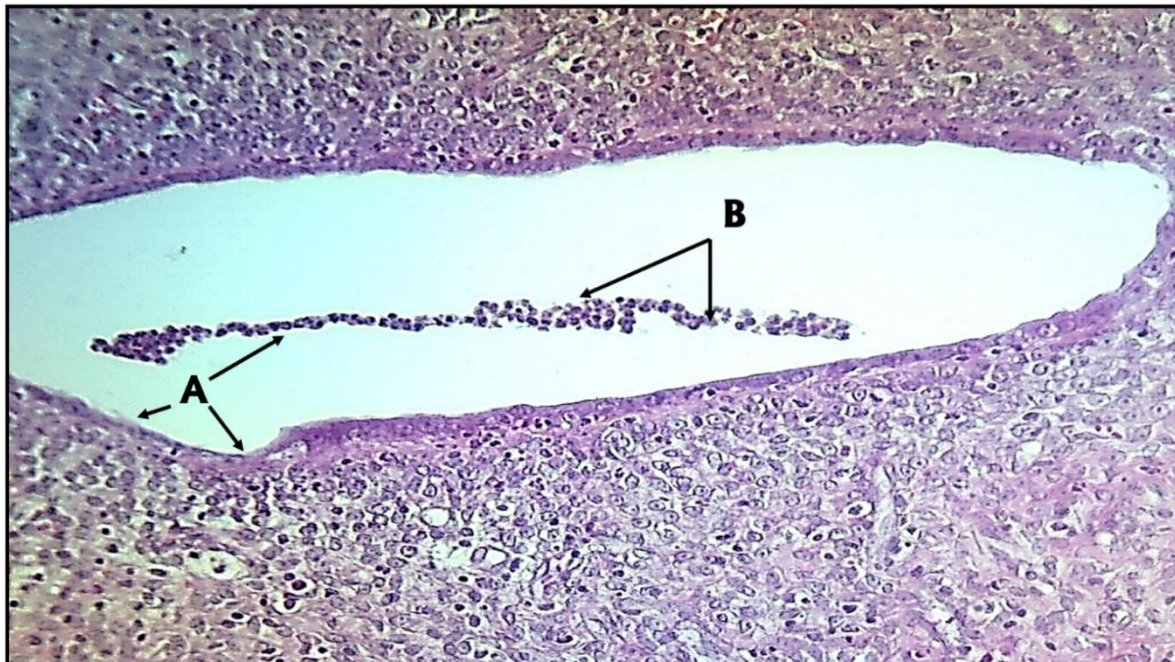


Figure 2: Micrograph of the uterus of a pregnant female rat infected with *C. albicans*, with rupture of smooth muscle fibers in the circular muscle layer (A) and irregular arrangement in the longitudinal muscle layer (B). (H&E, X40).

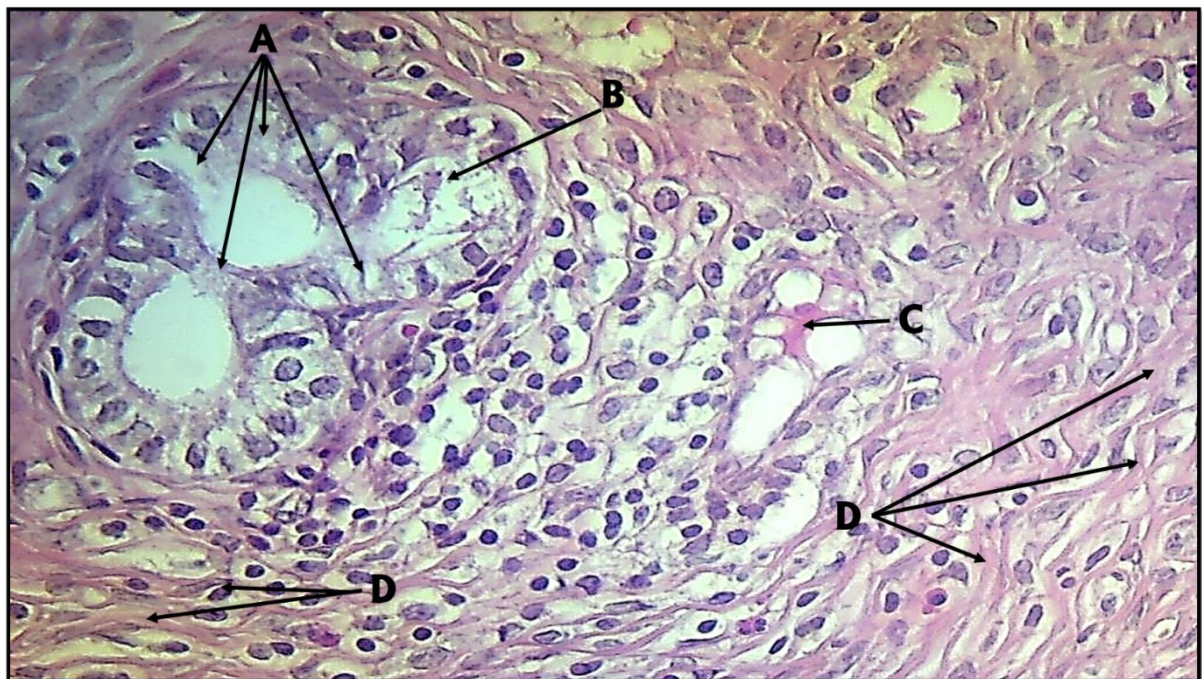
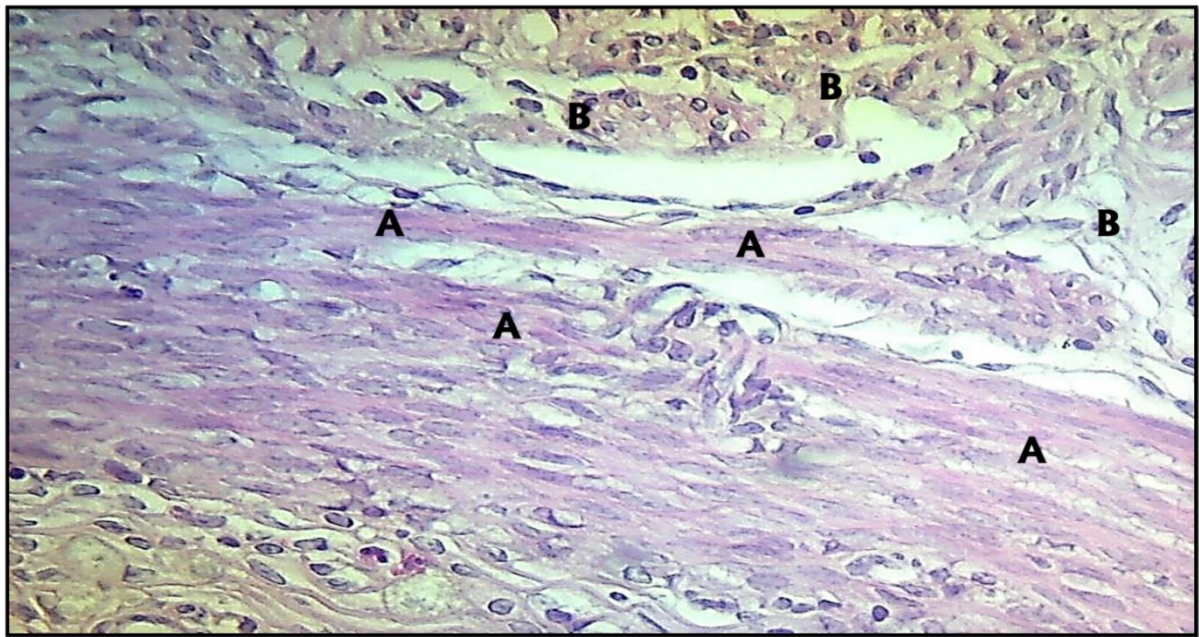


Figure 3: Micrograph of the uterus of a mouse from a group of pregnant women experimentally infected with *C. albicans*, in which necrosis of the lining epithelium of the uterine glands (A), some of which extended to the gland lumen (B), fibrous-edema of dilated blood vessels (C), collagen fibers (D). (H&E, X40).

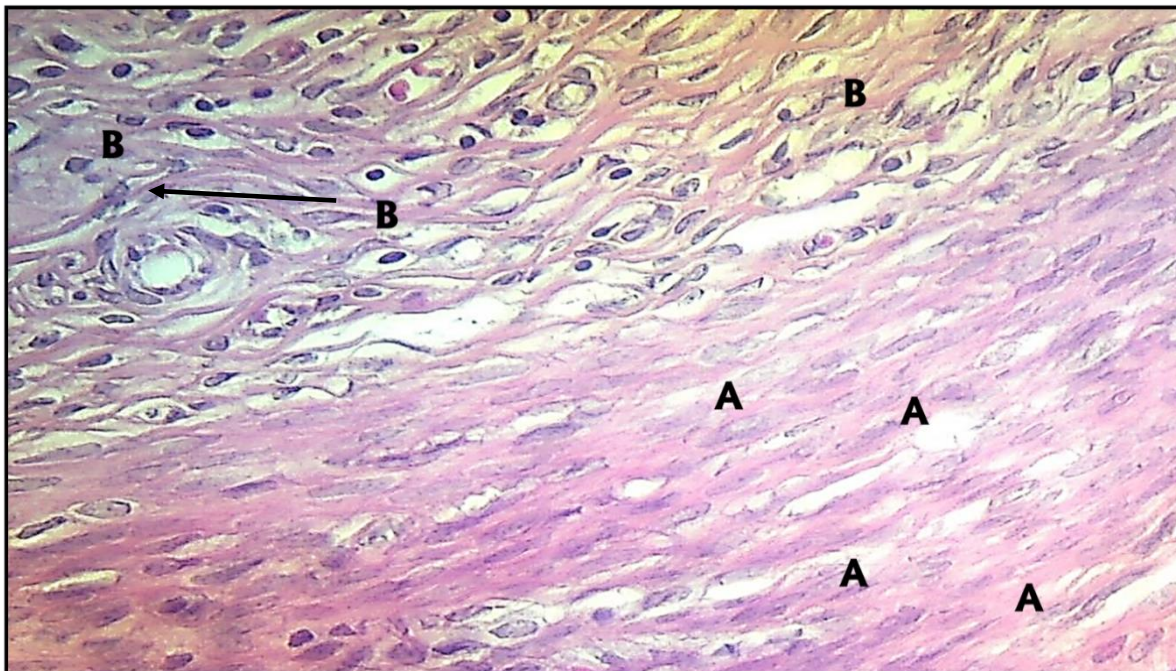


Figure 4: Micrograph of the uterus of a female mouse from a pregnancy group experimentally infected with *C. albicans*, showing a large flare-up of smooth muscle fibers in the circular layer (A), endometrial fibrosis (B). (H&E, X40).

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