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# Colonization of Mouse Reproductive Organs Following Vaginal Inoculation with Human Ureaplasma: Effect on Fertility

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**Abstract:** Ureaplasmas have been associated with infertility and poor pregnancy outcomes in humans; and to study this experimentally, twelve (12) adult female albino mice (in 4 groups) were inoculated intravaginally with 3 different dilutions of a human ureaplasma strain. Three (3) vaginal swabs taken from each of the animals before and after inoculations were used for cytological and bacteriological assessments. The animals were mated with males and observed for outcomes. Giemsa-stained vaginal smears revealed no difference in the quantity of epithelial cells before and after inoculation and no significant difference were observed between the numbers of Polymorphonuclear leucocytes (PMNL) seen 3 and 7 days after inoculation in any of the animal groups ($\chi^2$>0.05). Only 2 (28.6%) of the animals were colonized and organisms were recovered on sacrifice from the vagina of one and the vagina/uterus of the other. One (50.0%) of the colonized animals was not pregnant while the other had only 2 litters. The control animals were pregnant as well as 66% of the inoculated but un-colonized animals. Despite low colonization rate, absence of pregnancy and production of reduced litter sizes existed in colonized animals. The role of ureaplasmas in the vagina of women therefore needs more investigation.

**Key words:** Ureaplasma · mice · vaginal colonization · litter sizes

## INTRODUCTION

The ureaplasmas are members of the class of microorganisms called the Mollicutes. Human ureaplasmas colonize the genital tracts of adults and have been implicated in male and female infertility [1, 2]. Their presence in the female reproductive tract has been reported to lead to poor pregnancy outcomes [3] and other pregnancy associated disorders in gravid mothers [4].

To actually establish their pathogenic role in human genital tract infections, infertility and reproduction; ureaplasma strains have been inoculated into various experimental animals including man. Two human volunteers inoculated intraurethrally with *U. urealyticum* developed urethritis [5]. Animal models used by various workers include chimpanzees [6] Wistar rats [7] as well as several experiments involving intravaginal inoculation of mice [8, 9].This experiment with mice model was carried out to observe what effect a ureaplasma strain isolated from a woman with primary infertility may have in the reproductive tracts and consequently on the fertility of susceptible female mice when mated with male mice

## MATERIALS AND METHODS

**Mice:** Sixteen (16) locally bred adult albino mice (12 females and 4 males) aged between 8-10 weeks and obtained from the experimental animal unit of the Department of Veterinary Physiology and Pharmacology, University of Ibadan, Nigeria were used. The animals were kept in a well-aerated room in cages that were regularly cleaned. They were fed a pelleted diet and water *ad libitum*. The female mice were divided into groups (A to D) of three each with each mouse clearly identified with body marks/labels as $A_1$, $A_2$, $A_3$; $B_1$, $B_2$, $B_3$, etc. Groups A to C were used for the test, while group D was used as control.

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**Protocol:** One week before inoculation, the female mice were screened by collecting 3 swabs each from their vaginal tracts - one each was smeared on clean slides and stained by Giemsa to detect the presence of epithelial cells and polymorphonuclear leucocytes (PMNL). The 2nd and 3rd swabs were used to screen the animals for the presence of Mycoplasma/Ureaplasma. The medium used for the cultivation of genital mycoplasma is the modification of Modified Hayflick medium [10]. This medium was modified for ureaplasma cultures by incorporating 20% urea and thallium acetate was however omitted. The liquid-to-solid culture technique was employed whereby the swab samples first inoculated into mycoplasma and ureaplasma broths were subcultured onto their corresponding agar medium. The broth media were incubated in air at 37°C. The ureaplasma broths were incubated for 24 hours before being subcultured, the mycoplasma broths were incubated for up to 3 days before being subcultured. Incubation of the agar plates was in candle jar at 37°C. Using the dissecting microscope; the ureaplasma agar plates were examined after 24/48 hours while the mycoplasma plates were examined as from the third day and every 2 days for up to 10 days after which negative plates were discarded. Presence of typical mycoplasma/ureaplasma colonies showing "fried-egg" appearance were sought for and noted if present.

**Preparation of colony-forming unit/ml:** A ureaplasma strain obtained from a female patient with primary infertility (author's data) was inoculated into ureaplasma broth and after 48 hours incubation in air at 37°C, the broth was centrifuged at 2000 g for 30 minutes. The supernatant was discarded and 0.1 ml of the well-mixed deposit was added to 0.9 ml of ureaplasma broth to make a 1 in 10 dilution. From this, other 10-fold dilutions were made ($10^2$, $10^3$, up to $10^8$). These dilutions were subsequently cultured on ureaplasma agar to determine the number of organisms present in 1.0 ml of the original sample.

**Inoculation, sampling and sample processing:** 0.05 ml of each of 3 dilutions - $10^2$, $10^3$ and $10^4$ were used to inoculate the vagina of the mice in Groups A, B and C, respectively and the same volume of sterile broth was inoculated into the vagina of the control group. On days 3 and 7 post inoculation, 2 vaginal swabs were collected from each of the mice. One swab was inoculated into ureaplasma broth, incubated aerobically at 37°C and subsequently subcultured into its corresponding solid medium for possible recovery of organisms. The second swab was smeared on a clean slide, stained by Giemsa and examined for the presence of epithelial cells as well as PMNL.

**Mating:** One male mouse was introduced into each of the test and control groups after the 2nd sample collection on day 7, and allowed to mate. Observation was done on daily basis for pregnancies, miscarriages and preterm deliveries. On delivery, the litter sizes were counted and their weights recorded.

**Sacrifice:** The female mice were sacrificed 4 weeks after the males were introduced into them. The vagina and uterus were harvested from each animal. Likewise, samples of blood were drawn from their hearts

**Histopathology:** The kidney, liver, heart and lungs were also collected into formalin, processed and examined for histological findings.

**Statistical analysis:** All data were reported as simple percentages and the Chi square was used to test for significance.

## RESULTS

The screening result revealed that none of the animals harboured mycoplasma or ureaplasma in their vagina prior to inoculation. Table 1 shows types and number of cells obtained from the Giemsa stained smears of the vaginal samples before and after inoculation with ureaplasma. Chi square analysis showed that there was no significant difference between the numbers of PMNL seen 3 and 7 days after inoculation in any of the animal groups ($p = 0.098$; $p = 0.526$; $\chi^2 > 0.05$).

Table 2 shows the mating result of the animals and the litter sizes/litter weights obtained from each animal. On autopsy, it was observed that organisms were not recovered from any of the blood samples. However, two animals one each from Groups A and B (ie. $10^2$ and $10^3$ dilutions) were colonized. The mouse from group A had ureaplasmas in its vagina and that from group B in its vagina and uterus. Organisms were not recovered from any of the other mice on sacrifice (Table 3).

Histopathologically, apart from the pulmonary congestion found in one of the control mice, no lesions were observed from any of the other organs examined.

Table 1: Cells seen from the Giemsa stained vaginal smears before and after inoculation

| Animal groups | No. of cells seen 7 days before inoculation | | No. of cells seen after inoculation | | | |
|---|---|---|---|---|---|---|
| | | | Day 3 | | Day 7 | |
| | EC | PMNL | EC | PMNL | EC | PMNL |
| A1 | 2 | 0 | 3 | 0 | 6 | 0 |
| A2 | 3 | 0 | 2 | 0 | 5 | 1 |
| A3 | 0 | 0 | 0 | 0 | 2 | 1 |
| B1 | 0 | 0 | 0 | 0 | 1 | 2 |
| B2 | 0 | 0 | 0 | 0 | 2 | 0 |
| B3 | 2 | 0 | 0 | 3 | 1 | 2 |
| C1 | 2 | 0 | 0 | 0 | 2 | 0 |
| C2 | 0 | 0 | 0 | 0 | 1 | 1 |
| C3 | 0 | 0 | 2 | 3 | 3 | 3 |
| D1 | 2 | 0 | 1 | 0 | 0 | 0 |
| D2 | 2 | 0 | 2 | 0 | 0 | 1 |
| D3 | 1 | 0 | 1 | 0 | 0 | 0 |

Key: E.C - Epithelial cells, PMNL - Polymorphonuclear leucocytes

Table 2: Mating result of the mice

| Animal groups | Litter size | Average litter weights (g) |
|---|---|---|
| A1 | 2 | 1.65 |
| A2 | 8 | 1.34 |
| A3 | 8 | 1.31 |
| B1 | 13 | 1.23 |
| B2 | NP | - |
| B3 | 9 | 1.22 |
| C1 | NP | - |
| C2 | 11 | 1.22 |
| C3 | NP | - |
| D1 | 7 | 1.35 |
| D2 | NP | - |
| D3 | 5 | 1.52 |

Key: NP - Not pregnant

Table 3: Culture result of animal organs on sacrifice

| Mice groups | Blood | Vagina | Uterus |
|---|---|---|---|
| A1 | - | + | - |
| A2 | - | - | - |
| A3 | - | - | - |
| B1 | - | - | - |
| B2 | - | + | + |
| B3 | ND | ND | ND |
| C1 | ND | ND | ND |
| C2 | - | - | - |
| C3 | - | - | - |

Key: + = Growth, - = No growth, ND = Not done

## DISCUSSION

The lack of difference in the quantity of epithelial cells in this study before and after inoculation is similar to the finding of other workers who also reported no evidence of increase in number of epithelial cells after ureaplasma infection of chimpanzees' urethra [6]. Polymorphonuclear leucocytes (PMNL) were scanty in smears even after the animals were inoculated. Of interest though were 3 inoculated animals with no polymorphonuclear leucocytes even on the 7$^{th}$ day. Of these 3 mice, 2 (66.6%) were not pregnant at all while the 3$^{rd}$ gave birth to 2 litters only. Culture results of the mouse blood, vagina and uterus on sacrifice also revealed that ureaplasmas were recovered from 2 of these animals negative for leucocytes while the 3$^{rd}$ animal was lost before sacrifice and so had no samples cultured. The presence of leucocytes in the vaginal smears may have contributed to the non-susceptibility of the animals to ureaplasmas while its absence in the susceptible animals might have led to their susceptibility. In a similar way, other investigators recovered *U. urealyticum* from mice with scanty or no PMNL [8]. These authors thus concluded that a vaginal PMNL response to colonization by ureaplasmas and indeed, other mycoplasmas does not occur in mice. This they reported may be due to the effect of the hormone oestradiol that they administered to the mice to induce the oestrous phase of the reproductive cycle in which there

were few or no PMNL in vaginal smears. This treatment, they further reported, rendered the mice susceptible to genital tract colonization by serotype 8 of *U. urealyticum* inoculated intravaginally as against the untreated mice that were resistant. The result from this present study thus shows that the organism did not colonize 71.4% of the mice and the 28.6% that were colonized could have been in a reproductive phase when susceptibility was most likely.

Culture results showed that organisms were not recovered from any of the animals prior to mating and in the susceptible animals; it was recovered only on sacrifice. The strain of mice used in this study could have been responsible for the poor colonization of the ureaplasma. The local albino mice obtainable in this country were used and their susceptibility to ureaplasma infection had not been previously tested. The BALB/C strain out of several strains of female adult mice has been reported to be the most susceptible to vaginal colonization by *Ureaplasma urealyticum* [8].

Ureaplasmas were not isolated from the blood samples of any of the animals. This is not unexpected, as the organism has not been known to be invasive [11] however they were recovered from the vagina and even the uterus of one of the animals. In a reported study, ureaplasmas were recovered from the vagina and some spread to the uterine horns, ovaries and even spleen in one mouse [8]. In a later study, these authors reported that dissemination of ureaplasmas beyond the genital tract is likely to be strain-related, as this trait was not demonstrated in the SCID mice but in the BALB/C mice [9]. The histopathology result in this study did not reveal any lesion in the organs studied thus further supporting the non-colonizing status of the majority of the mice used.

Only slight difference was observed between the average litter sizes/birth weights of the inoculated and the control mice. In a mating experiment with rats, a smaller mean litter size and a lower birth weight were observed in the offspring's of infected males compared with the control animals [7]. However, they reported no influence on the fertility of infected animals. Majority of the animals in this study were not colonized and so the effect of ureaplasmas in their vaginal tract could not be studied. However, the fact that of the 2 colonized mice, 1 (50.0%) was not pregnant while 1 (50.0%) produced reduced litter size could suggest a possible role of this organism in both infertility and production of few litter size. It was also observed that there was no pregnancy in one third (33.3%) of the control mice. Since the control mice were not inoculated, it will be assumed that this lack of pregnancy in one of them might have been due to ill health as a result of the pulmonary congestion found in its lung. In view of these findings, it is thus being suggested that colonization of ureaplasma may lead to the absence of pregnancy or production of reduced litter sizes in colonized animals. The role of ureaplasmas in human infertility is therefore worth more investigation.

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