INTRAVAGINAL INOCULATION OF THE GUINEA-PIG WITH *VIBRIO FETUS* VAR. *VENEREALIS* DURING NATURAL AND INDUCED REPRODUCTIVE CYCLE PHASES*

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Summary. Trials were conducted in which virgin female guinea-pigs were inoculated intravaginally with *Vibrio fetus* var. *venerealis* during the follicular and luteal phases of the reproductive cycle, during induced and natural oestrus and following the intramuscular injection of progesterone. In all trials, the results gave evidence of transitory infection, but failed to indicate any gross or microscopic changes in the reproductive system attributable to *V. fetus* var. *venerealis* infection.

INTRODUCTION

Smith (1918) reported the guinea-pig to be refractory to *Vibrio fetus* infection, and later (Smith, 1919) extended this view to include most small animal species used in laboratory studies. When the intraperitoneal route of inoculation was used, however, Smith (1923) was successful in infecting two out of three female guinea-pigs.

Ristic & Morse (1953) inoculated pregnant guinea-pigs by various routes, with a mixed culture of ovine and bovine isolants of *V. fetus*, and reported infection in one guinea-pig inoculated intraperitoneally. Adler (1953) inoculated oestrogen-primed non-pregnant guinea-pigs with pure cultures of *V. fetus* by the vaginal route. The same author (Adler, 1953) included animals inoculated intravaginally with infected bull semen. A 50% infection rate was recorded in the test animals. A report by Power (1954) indicated almost identical results.

In the present study, attempts were made to establish infection with *V. fetus* var. *venerealis* (as defined by Florent, 1963) in the non-pregnant guinea-pig by inoculation into the vagina. The experimental conditions were varied so that infection was attempted during (1) the follicular and luteal phases of the reproductive cycle, (2) induced and natural oestrus and, (3) after the administration of exogenous progesterone.

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MATERIALS AND METHODS

Vibrio isolant

The organism used was a fresh smooth isolant of *V. fetus* var. *venerealis* previously identified by the criteria of Florent (1963). To avoid repeated subculture, stock cultures of the isolant were grown in Brucella broth (Albimi) for 24 to 48 hr at 35°C and stored at -70°C. The techniques of isolation and propagation were those of Lovell (1964), and the gas mixture used for the growth of the isolants was that of Lovell (1964) as modified by Walsh & White (1968).

Experimental animals

Mature virgin female guinea-pigs of 450 to 500 g weight were used. The animals were of the mixed and English short hair varieties. Each guinea-pig was housed separately in quarters maintained at $68 \pm 5^\circ\text{F}$. They were fed a diet of Purina guinea-pig chow supplemented with hay and fresh cabbage twice weekly and allowed free access to water. Reproductive cycle phases were determined from examination of the vaginal cytology as described by Asdell (1946).

Experiment 1. Inoculation during the follicular and luteal phases of the reproductive cycle

Eight guinea-pigs were placed into two groups, four in the follicular and four in the luteal phase of the reproductive cycle. One animal from each group served as a control. Vaginal cultures were made from each animal to establish the existence of a vibrio-free status. A blunt medicine dropper was used to inoculate the animals intravaginally. Each animal received approximately 1×10^7 viable cells of *V. fetus* in Brucella broth on each of 3 successive days. The control animals were given an equal volume of Brucella broth only. Before the administration of the inoculum on the 2nd and 3rd days, a vaginal culture and smear were taken. Cultures were made for 3 consecutive days after the final inoculation. All the animals were killed 5 days after the final inoculation. The entire reproductive tract was removed aseptically. Cultures were taken from the vagina, proximal and distal cervical areas, both uterine horns and ovaries, the liver, spleen and heart blood. Portions of each tissue were cultured in thioglycollate broth (Difco). Uterine and cervical tissues were preserved in 10% formalin for subsequent sectioning and staining with haematoxylin and eosin.

Experiment 2. Inoculation during induced and natural oestrus

Six test and two control animals were used. Their vibrio-free status was confirmed. The control animals were allowed to reach oestrus naturally. The induced oestrous period of the test animals was synchronized with that of the controls by two intramuscular injections of 300 i.u. oestradiol benzoate (Pro-gynon benzoate, Schering Corp., Bloomfield, N.J.) given 72 hr apart. The techniques of inoculation and subsequent examination were identical to those described in Exp. 1. The inoculum given comprised 3×10^5 viable cells per dose to each guinea-pig in the trial.

Experiment 3. Inoculation of animals treated with progesterone

The vibrio-free status of six test and two control guinea-pigs was established. Each test animal was given 2.5 mg progesterone (repository type, 25 mg/ml) intramuscularly daily for 3 days. After the third injection, inoculation of both test and control animals was started. The size of each dose of inoculum was 3×10^6 viable cells. The study was completed with the methods described in Exp. 1.

RESULTS

Experiment 1. Inoculation during follicular and luteal phases

All animals were vibrio-free before inoculation. None showed any adverse physiological response to the inoculum and remained active and alert throughout the experimental procedures. Anorexia was not noted. The cellular response observed in the vagina of the normally cyclic animals reflected the hormonal state of the reproductive system. Vaginal cytology revealed a slight increase in neutrophils which became more extensive on the 3rd day after

Table 1. Growth of *V. fetus* in vaginal cultures obtained from guinea-pigs during and after intravaginal inoculation

Phase of cycle	Animal no.	During inoculation*		After inoculation			Necropsy 5 days after inoculation†
		24 hr	48 hr	24 hr	48 hr	72 hr	
Test animals	1	+	+	+	+	+	—
	2	+	+	+	—	—	—
	3	+	+	+	+	—	—
	4	+	+	+	+	—	—
	5	+	+	+	—	+	—
	6	+	+	+	+	—	—
Control animals	7	—	—	—	—	—	—
	8	—	—	—	—	—	—

+, Growth of *V. fetus*; —, no growth of *V. fetus* in 5 days at 37°C.

* Cultures taken 24 and 48 hr after Day 1 of the inoculation period.

† No isolations of *V. fetus* were made from the uterus, cervix, blood, liver or spleen.

inoculation. At necropsy, no gross lesions were observed in the reproductive tract or other visceral organs. Histologically, the uterine tissue of the test animals showed a slight inflammatory reaction with some congestion of the lamina propria. No differences in the gross and microscopic appearances of tissues examined from animals in the luteal or follicular phase of the reproductive cycle could be attributed to the inoculation of *V. fetus*. The results of the bacteriological findings are given in Table 1. Similar patterns of *V. fetus* isolation occurred in both test groups. All animals yielded positive cultures 24 hr after inoculation. After this period, isolations of *V. fetus* from both groups declined, two of three in 48 hr and one of three in 72 hr. All tissues cultured at necropsy 5 days after inoculation were free of *V. fetus*.

Experiment 2. Inoculation during induced and natural oestrus

The animals inoculated during induced and natural oestrus were showing characteristic cornification of the vaginal epithelial cells. Those treated with exogenous oestrogen appeared to tolerate the hormone well. A marked increase in neutrophils was observed in the vaginal cytology. No gross or microscopic lesions were observed at necropsy. The pattern of *V. fetus* isolation is given in Table 2. In animals during both induced and naturally occurring oestrus the infection persisted only throughout the inoculation period, becoming negative within 24 hr after inoculation. Tissues cultured at necropsy were free of *V. fetus*.

Table 2. Growth of *V. fetus* in vaginal cultures obtained from guinea-pigs during and after intravaginal inoculation during oestradiol-induced and natural oestrus

	Animal no.	During inoculation*		After inoculation			Necropsy 5 days after inoculation†
		24 hr	48 hr	24 hr	48 hr	72 hr	
Test (oestradiol-induced oestrus)	1	+	+	—	—	—	—
	2	+	+	—	—	—	—
	3	+	+	—	—	—	—
	4	+	+	—	—	—	—
	5	+	+	—	—	—	—
	6	+	+	—	—	—	—
Control (natural oestrus)	1	+	+	—	—	—	—
	2	+	+	—	—	—	—

+, Growth of *V. fetus*; —, no growth of *V. fetus* in 5 days at 37°C.

* Cultures taken 24 and 48 hr after Day 1 of the inoculation period.

† No isolations of *V. fetus* were made from the uterus, cervix, blood, liver or spleen.

Table 3. Growth of *V. fetus* in vaginal cultures obtained from progesterone-treated guinea-pigs during and after intravaginal inoculation

	Animal no.	During inoculation*		After inoculation			Necropsy 5 days after inoculation†
		24 hr	48 hr	24 hr	48 hr	72 hr	
Test (progesterone)	1	+	+	—	—	—	—
	2	+	+	—	—	—	—
	3	+	+	+	—	—	—
	4	+	+	—	—	—	—
	5	+	+	+	—	—	—
	6	+	+	—	—	—	—
Control (no progesterone)	7	+	+	+	—	—	—
	8	+	+	—	—	—	—

+, Growth of *V. fetus*; —, no growth of *V. fetus* in 5 days at 37°C.

* Cultures taken 24 and 48 hr after Day 1 of the inoculation period.

† No isolations of *V. fetus* were made from the uterus, cervix, blood, liver or spleen.

Experiment 3. Inoculation of animals treated with progesterone

Following treatment with progesterone, guinea-pigs manifested tenderness and stiffness at the site of injection. A marked eosinophilia was noted in the vaginal cytology of the test animals. This persisted throughout the study. A

polymorphonuclear response was seen in both test and control animals during the inoculation period. No gross or microscopic lesions were present in either group at necropsy. *Vibrio fetus* was isolated only during the inoculation period from both test and control animals (Table 3). Two of six test and one of two control animals gave positive cultures 24 hr after inoculation. Tissues cultured from both test and control groups at necropsy were negative for *V. fetus*.

DISCUSSION

The *in-vivo* trials described are apparently the first to use a defined isolant of *V. fetus* var. *venerealis* in the study of guinea-pig infection with the organism generally known as *Vibrio fetus*. The vaginal route of inoculation was used because it more closely resembles the normal venereal route of infection in cattle, although Ristic & Morse (1953) reported intravaginal inoculation as the least effective method for the establishment of infection in guinea-pigs. Tables 1 to 3 show a lack of persistence of *V. fetus* in the vagina even when introduced in large numbers. This is in direct contrast with the infection in the heifer in which VanDerplasse, Florent & Huysman (1957) reported a persistence of at least 8 months' duration.

The bacteriological and histological examinations made after intravaginal inoculation of the guinea-pig gave no indication that the organism entered the uterus. The possibilities of uterine invasion of a duration of less than 5 days was not ruled out in this study. The value of exogenous oestrogen to increase the susceptibility of the guinea-pig to *V. fetus* infection as described by Power (1954) and Adler (1953) was not confirmed in this study. A lack of persistence of the vaginal infection was noted with some indication that the rate of clearance from the vagina was even greater in oestrous animals, regardless of whether oestrus was induced or not. The results of this work support the findings of Ristic & Morse (1953) and Robinson, van Rensburg, van Heerden & van Drimmelen (1956) who rejected intravaginal inoculation in favour of intra-peritoneal inoculation in guinea-pig studies, especially if the animals were used for diagnostic purposes.

Exogenous progesterone apparently did not increase the susceptibility of the guinea-pig to *V. fetus* infection following intravaginal inoculation. The results of these studies again reflected the lack of persistence described above under other experimental conditions. It is of interest to note that Manclark & Pickett (1965) were unable to detect the formation of either mucosal or humoral antibodies in the guinea-pig after intravaginal inoculation with live cells of *V. fetus*. Both humoral and mucosal antibodies were found in high titre when live *V. fetus* cells were inoculated intraperitoneally into mated but non-pregnant guinea-pigs. The failure of the immune response in animals inoculated intravaginally may reflect the apparent lack of antigen persistence demonstrated throughout this work.

Under the experimental conditions used, it is apparent that the female guinea-pig is not a reliable test animal for the diagnosis of *V. fetus* infection in the cow, especially if the intravaginal route of inoculation is used. The cervix apparently forms an effective barrier to the entry of *V. fetus* to the guinea-pig

uterus and precludes the development of systemic infection. The refractory response of the guinea-pig is considered to be due to the strict host specificity of *V. fetus* var. *venerealis* which allows for the fullest expression of its virulence solely in the reproductive tract of the cow.

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