

## Effects of intrauterine infection by *Staphylococcus aureus* and *Mycoplasma capricolum* on the fertility of Nubian goats

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**Summary.** Oestrous cycles were followed by blood progesterone titration and the occurrence of pregnancy was observed after mating in three groups of mature female Nubian goats. Two groups of 4 and 3 goats each were experimentally infected by an *in utero* injection of either a *Staphylococcus aureus* or a *Mycoplasma capricolum* strain of caprine origin. A third group of 4 goats served as a control. Both bacterial infections persisted in all experimental animals, except one, for at least 70 days. As a result, there is a shortening of the first two cycles and progesterone concentration was lower than in the controls. Only one inoculated goat became pregnant after the third oestrus. It is suggested that bacterial inflammation of the uterus may thus modify the oestrous cycle through the prostaglandin pathway.

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### Introduction.

Bacterial infection of the female genital tract, giving rise to bacterial toxins and inflammation, may influence the reproductive functions. It is well known that some Gram-negative infections cause abortion in domestic animals (Roberts, 1971). Experimental intrauterine infections at different stages of the luteal phase also affect the length of the oestrous cycle (Brinsfield *et al.*, 1969). The purpose of this study was to investigate the effect of *Staphylococcus aureus* and *Mycoplasma capricolum* on corpus luteum regression and its consequences on reproductive functions in female Nubian goats.

### Material and methods.

Eleven mature 3 to 4-year old female Nubian goats with a normal oestrous cycles (19 to 21 days) were used. Oestrous (Day 0) was synchronized in all the

goats by intramuscular injection of two doses (8 mg each) of prostaglandin  $F_{2\alpha}$  (Luteolyse, Upjohn, USA) 10 days apart. The animals came into oestrus 24 to 48 h after the second injection and were then divided randomly into three groups. Group I (nos. 1008, 1012, 1023 and 1028) included 4 goats used as an untreated control. Group II (nos. 1007, 1009, 1016 and 1018) included 4 goats inoculated with 1 ml ( $18 \times 10^8$  organisms) of *Staphylococcus aureus* (G 27 strain) by intrauterine route on Day 0 ; the bacterium was obtained from washing the uterus of goats in the Kassala Veterinay Clinic, Sudan. Group III (nos. 1029, 1032 and 1024) included three goats inoculated with 1 ml ( $10^9$  CFU) of *Mycoplasma capricolum* (O 37 strain) via intrauterine route ; this strain was obtained from the endometrial scrapings of sheep from the Omdurman slaughter house, Sudan.

Blood samples, obtained every two days by venipuncture using 23 G needles and collected into chilled tubes, were centrifuged at  $1\ 800 \times g$  for 10 min ; the plasma was separated and stored at  $-30\ ^\circ\text{C}$  until analysed for progesterone by radioimmunoassay (RIA). Weekly uterine washings were taken from the goats to reisolate the bacteria. Uterine washing was stopped for a complete cycle (21 days) after mating and resumed in those which failed to get pregnant. The goats were observed for one complete cycle. When they returned to oestrus, a fertile buck was introduced, mating was permitted (except for goats nos. 1008 and 1012 in Group I), and the goats were observed for 10 weeks.

## Results.

The results are summarized in tables 1 and 2. *S. aureus* and *M. capricolum* were reisolated from Groups II and III, respectively, in the first week after inoculation and thereafter until week 3 in goat 1007, week 8 in goats 1016 (Group II) and 1032 (Group III) and up to week 10 in the rest of the animals. No bacterium was isolated from the controls (Group I) ; goats 1023 and 1028 were checked for bacterium until week 3.

Peripheral plasma concentrations of progesterone in Group I are given in table 2. All the goats had a functional corpus luteum, as shown by the progesterone levels which were about 3.5 ng/ml on Day 7 and 4.4 ng/ml on Day 14 of the oestrous cycle. On Days 20 to 21 of the oestrous cycle, all the animals returned to oestrus. The signs of oestrus were tail fanning, restlessness and acceptance of the male ; progesterone concentration was  $< 1$  ng/ml. The male was introduced on that day. Progesterone concentration on Days 7 and 14 in the second oestrous cycle was similar to that in the first cycle and goats nos. 1023 and 1028 did not return to oestrus. They were confirmed to be pregnant by their progesterone concentrations which were about 4 ng/ml on Day 22 of the second oestrous cycle (Day 42 of the experiment). Goats nos. 1008 and 1012 returned to oestrus 42 days after the experiment.

Peripheral plasma concentrations of progesterone in Groups II and III are summarized in table 1. Three days after inoculation, the progesterone level increased to 1.5 ng/ml and then dropped suddenly to less than 1 ng/ml on Day 7 or 10 of the cycle. Only goats 1007 (Group II) and 1032 (Group III) exhibited behavioural oestrus. The second oestrous cycle was also shortened in these

TABLEAU 1

Progesterone plasma concentrations (ng/ml  $\pm$  SD) in control or inoculated goats.

Days post inoculate	Controls (a) goat n°					Saphylococcus (b) inoculated goat n°					Mycoplasma (c) inoculated goat n°					
	1008	1012	1023	1028	1007	1009	1016	1018	1029	1024	1009	1016	1018	1029	1032	1024
3	3.9 $\pm$ 0.6	3.6 $\pm$ 0.5	3.9 $\pm$ 0.3	—	1.6 $\pm$ 0.1	2.1 $\pm$ 0.4	1.5 $\pm$ 0.2	2.0 $\pm$ 0.3	1.6 $\pm$ 0.3	1.8 $\pm$ 0.3	—	—	—	—	2.1 $\pm$ 0.3	1.8 $\pm$ 0.3
7	3.9 $\pm$ 0.6	3.6 $\pm$ 0.5	3.9 $\pm$ 0.3	3.1 $\pm$ 0.3	3.2 $\pm$ 0.4	3.5 $\pm$ 0.4	0.4 $\pm$ 0.1	0.5 $\pm$ 0.2	3.3 $\pm$ 0.4	0.4 $\pm$ 0.2	0.6 $\pm$ 0.2	0.4 $\pm$ 0.1	0.5 $\pm$ 0.2	3.3 $\pm$ 0.4	0.6 $\pm$ 0.2	0.4 $\pm$ 0.2
10	—	—	—	—	0.6 $\pm$ 0.2	0.5 $\pm$ 0.2	1.4 $\pm$ 0.3	1.5 $\pm$ 0.3	0.6 $\pm$ 0.3	1.1 $\pm$ 0.2	—	—	—	1.1 $\pm$ 0.2	1.5 $\pm$ 0.3	1.5 $\pm$ 0.3
14	4.6 $\pm$ 0.6	4.8 $\pm$ 0.4	4.3 $\pm$ 0.4	4.6 $\pm$ 0.4	2.0 $\pm$ 0.4	2.2 $\pm$ 0.3	2.5 $\pm$ 0.4	2.6 $\pm$ 0.3	1.5 $\pm$ 0.2	2.0 $\pm$ 0.4	—	—	—	2.0 $\pm$ 0.4	2.5 $\pm$ 0.4	2.5 $\pm$ 0.4
21	0.5 $\pm$ 0.2	0.4 $\pm$ 0.2	0.3 $\pm$ 0.2	0.5 $\pm$ 0.6	0.4 $\pm$ 0.2	2.6 $\pm$ 0.4	2.8 $\pm$ 0.3	2.6 $\pm$ 0.3	2.7 $\pm$ 0.3	3.2 $\pm$ 0.5	—	—	—	3.2 $\pm$ 0.5	3.3 $\pm$ 0.4	3.3 $\pm$ 0.4
28	3.2 $\pm$ 0.4	3.5 $\pm$ 0.4	3.4 $\pm$ 0.4	3.1 $\pm$ 0.3	2.8 $\pm$ 0.3	0.6 $\pm$ 0.1	2.8 $\pm$ 0.4	0.5 $\pm$ 0.2	2.6 $\pm$ 0.3	0.6 $\pm$ 0.2	—	—	—	0.6 $\pm$ 0.2	0.7 $\pm$ 0.2	0.7 $\pm$ 0.2
35	4.3 $\pm$ 0.3	4.6 $\pm$ 0.5	4.4 $\pm$ 0.5	5.1 $\pm$ 0.6	3.0 $\pm$ 0.4	1.7 $\pm$ 0.2	2.1 $\pm$ 0.3	1.8 $\pm$ 0.2	3.1 $\pm$ 0.3	1.4 $\pm$ 0.3	—	—	—	1.4 $\pm$ 0.3	1.9 $\pm$ 0.3	1.9 $\pm$ 0.3
42	0.6 $\pm$ 0.1	0.4 $\pm$ 0.3	4.8 $\pm$ 0.5	4.9 $\pm$ 0.5	4.6 $\pm$ 0.5	3.2 $\pm$ 0.3	0.5 $\pm$ 0.2	3.9 $\pm$ 0.4	0.4 $\pm$ 0.1	3.1 $\pm$ 0.4	—	—	—	3.1 $\pm$ 0.4	3.6 $\pm$ 0.4	3.6 $\pm$ 0.4
49	3.8 $\pm$ 0.4	3.1 $\pm$ 0.5	4.9 $\pm$ 0.6	5.1 $\pm$ 0.5	4.7 $\pm$ 0.5	0.6 $\pm$ 0.2	1.8 $\pm$ 0.2	0.5 $\pm$ 0.2	1.6 $\pm$ 0.2	0.5 $\pm$ 0.1	—	—	—	0.5 $\pm$ 0.1	0.7 $\pm$ 0.3	0.7 $\pm$ 0.3
56	4.5 $\pm$ 0.3	4.5 $\pm$ 0.5	4.9 $\pm$ 0.5	4.5 $\pm$ 0.4	4.2 $\pm$ 0.5	1.6 $\pm$ 0.3	3.1 $\pm$ 0.3	1.7 $\pm$ 0.3	3.1 $\pm$ 0.4	1.8 $\pm$ 0.3	—	—	—	1.8 $\pm$ 0.3	2.8 $\pm$ 0.3	2.8 $\pm$ 0.3
63	0.6 $\pm$ 0.2	0.5 $\pm$ 0.2	4.7 $\pm$ 0.4	4.8 $\pm$ 0.5	4.2 $\pm$ 0.5	3.6 $\pm$ 0.4	0.6 $\pm$ 0.1	3.1 $\pm$ 0.4	0.4 $\pm$ 0.1	3.2 $\pm$ 0.4	—	—	—	3.2 $\pm$ 0.4	2.6 $\pm$ 0.4	2.6 $\pm$ 0.4
70	3.3 $\pm$ 0.5	3.6 $\pm$ 0.4	4.7 $\pm$ 0.5	4.9 $\pm$ 0.5	4.6 $\pm$ 0.5	0.6 $\pm$ 0.2	1.4 $\pm$ 0.2	0.7 $\pm$ 0.2	1.2 $\pm$ 0.3	0.6 $\pm$ 0.2	—	—	—	0.6 $\pm$ 0.2	0.6 $\pm$ 0.2	0.6 $\pm$ 0.2

(a) : all bacterial samples negative (goats 1023 and 1028 checked for bacteria until week 3) ;

(b) : all bacterial sample positive except 1016 at day 70 (goat 1007 was checked for bacteria until week 3) ;

(c) : all bacterial samples positive except 1032 at days 63 and 70.

animals by at least 14 days. They all showed behavioural oestrus. The length of the third oestrous cycle was about normal (18 to 21 days). Goat 1007 (Group II) did not return to oestrus and was pregnant 42 days after inoculation; its progesterone concentration was 4.8 ng/ml on that day.

During the oestrous cycle, progesterone concentrations in Groups II and III were significantly ( $P < 0.01$ ) lower than in Group I.

## Discussion.

The present results demonstrate that the life span of the corpus luteum in goats is shortened when bacterial infection is induced in the uterus on the day of oestrus. Similar results were obtained in ewes (Brinsfield and Hawk, 1968a; Brinsfield, Higginbotham and Hawk, 1969). Such a mechanism apparently operates at least partly through the local utero-ovarian pathway (Brinsfield and Hawk, 1968b).

The effect of bacteria or bacterial toxins may be to cause inflammatory processes in the uterus. Prostaglandins have been found to be important mediators of inflammation (Veno *et al.*, 1981). Prostaglandins, and prostaglandin  $F_{2\alpha}$  specifically, are known to be luteolytic (Horton and Poyer, 1976). This may explain the shortening of the oestrous cycle in experimentally infected ewes and goats. Group II and III animals in the present study were observed to have significantly lower progesterone levels than the controls. This may be due to partial luteolysis resulting from the low release of prostaglandin  $F_{2\alpha}$  which was not sufficient to cause complete regression of the corpus luteum; it has been reported that prostaglandin release induced by bacterial toxins and the subsequent occurrence of oestrus are dose-dependent in ewes (Friedricksson, Kindahl and Edquist, 1985). The present results suggest that bacterial infection in the uterus may affect reproduction, provided that enough bacterial toxins and inflammatory processes result to induce sufficient prostaglandin synthesis and release that will cause luteal regression.

In cases of uterine infection this might be partially beneficial since the release of prostaglandin induces oestrus and thus contraction of the uterus and renewal of uterine cellular elements which can aid the healing process (Fredriksson, Kindahl and Edquist, 1985). Such a phenomenon may have occurred in goats 1016 and 1032 which recovered spontaneously. Although all infected goats showed short oestrous cycles, only one goat out of seven became pregnant. This may reflect reduced fertility in those animals. Studer and Morrow (1978) found a significant increase in the number of services per conception in infected cows, and Tennant and Peddicord (1968) showed a significant relationship between endometritis and decreased fertility in cows. This emphasizes the need to protect the uterus from bacterial contamination in order to avoid negative effects on the conception rate (Dekvuif and Brand, 1978).

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**Résumé.** *Effets d'une infection intrautérine, par Staphylococcus aureus et Mycoplasma capricolum, sur la fertilité des chèvres nubiennes.*

Le déroulement des cycles estriens de 3 groupes de chèvres nubiennes a été déterminé par dosage de la progestérogène plasmatique, de même que l'existence d'une gestation après accouplement. Au début de l'expérience les cycles ont été synchronisés par injection de PGF<sub>2α</sub>. Un premier groupe de 4 animaux a servi de témoin. Les 4 chèvres du 2<sup>e</sup> groupe ont été infectées expérimentalement par injection *in utero* d'une suspension de *Staphylococcus aureus* et les 3 chèvres du 3<sup>e</sup> groupe, par une souche de *Mycoplasma capricolum*. L'infection a persisté dans toutes les chèvres injectées, à l'exception d'une seule, pendant les 70 jours d'examen. Les chèvres infectées ont montré un raccourcissement de la durée des deux premiers cycles et un niveau de progestérogène significativement plus bas que celui des animaux témoins. Il est suggéré que ces changements peuvent être liés à la sécrétion de PGF<sub>2α</sub> par l'utérus. Une seule chèvre infectée est devenue gestante.

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