RELATION OF PLASMA PROGESTERONE TO MID AND LATE TERM BOVINE ABORTIONS DUE TO VIBRIO FETUS INFECTION

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Summary. The inoculation of viable cultures of Vibrio fetus into the uterus of cows in the second and third 3-monthly periods of pregnancy resulted in abortion. All foetuses exposed during the second 3 months were aborted 5 to 7 days post-inoculation; foetal death occurred several days before expulsion. Peripheral plasma progesterone levels declined at the time of foetal death.

Cows injected with V. fetus during the third 3 months of pregnancy aborted 9 to 20 days post-inoculation and in a majority of cases delivered live calves. The decline of progesterone levels on the day of abortion is very similar to that observed before normal parturition. Progesterone levels in the dam reflect the viability status of the foetus. The decline of progesterone associated with abortion may be due both to placental dysfunction as well as luteolysis of the corpus luteum because of the release of products from the infected foetus.

INTRODUCTION

There is a paucity of information available regarding the hormonal relationship to abortions associated with infectious diseases. The lack of techniques for accurately quantitating hormones associated with pregnancy in relatively small amounts of blood has contributed to this void. The development of a technique whereby a monochloroacetate derivative of progesterone was quantitated by gas–liquid chromatography with electron capture detection made it possible to monitor levels of progesterone in relatively small amounts (20 ml) of plasma (van der Molen & Groen, 1965; Stabenfeldt, Ewing, Patton & McDonald, 1969). The availability of this technique provided an opportunity to study progesterone levels in detail through multiple determinations in individual animals. This experiment was designed to study the relationship of peripheral plasma progesterone levels to the development of pathological changes (including abortion) caused by experimental infection of cows with Vibrio fetus.

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Eight cows without history of previous exposure to genital vibriosis were mated to a 2-year-old bull. Laparotomies were performed on the cows at designated stages of gestation (4, 5, 6, 7 and 8 months), and 1.5 ml of a 48-hr culture of a 10⁹ suspension of viable *Vibrio fetus*, variety *venerialis*, were injected into the foetal membranes. Sterile water (1.5 ml) was injected into the foetal membranes of the control animals. Cows were observed daily and in some cases examinations per rectum were performed to determine foetal viability. Aborted foetuses and placentae were recovered, cultured and examined for gross and microscopic lesions.

Approximately 1 to 5 days before inoculation and continuing for at least 3 days after foetal expulsion, 100 ml of blood were collected daily in 10 ml of 10% potassium oxalate. Progesterone determinations were performed in duplicate on 20-ml plasma samples as previously reported (Stabenfeldt, 1968; Stabenfeldt et al., 1969). Briefly, the method entails dichloromethane extraction of progesterone from plasma, saponification of the extraction residue, isolation of progesterone by thin-layer chromatography (TLC), enzymatic conversion of progesterone to 20β-hydroxypreg-4-en-1-one, acetylation with monochloro-acetic anhydride and quantitation by gas-liquid chromatography (GLC) with electron capture detection and liquid scintillation spectrometry. Identification of 20β-hydroxyprog-4-en-3-one chloroacetate (isolated as progesterone from plasma) was by the establishment of a constant specific activity in several TLC systems and by determination of its molecular weight through mass spectrometry (Waller, 1968).

RESULTS

Text-figure 1 is a diagram illustrating the effect of inoculation of *V. fetus* at various stages of gestation and the number of days which transpired before abortion occurred. Cows in the second 3 months of pregnancy aborted 6 days (average) after inoculation while cows in the third 3 months aborted 13 days (average) after inoculation.

The results presented in Text-fig. 2 show plasma progesterone levels in cows exposed to *V. fetus* during the 4th, 5th and 6th months of pregnancy. Progesterone levels varied between 3 and 6 μg/ml of plasma in all cows before inoculation. A precipitous decline of progesterone to less than 1 μg/ml of plasma was observed 3 to 7 days post-inoculation. Abortion occurred 1 to 3 days after the progesterone decline. Evidence of foetal viability ceased in the infected foetuses between the 2nd and 4th days post-inoculation or approximately 2 to 4 days before expulsion. Aborted foetuses had extensive autolytic changes. Gross placental lesions consisted of flaccid, tawny or grey-white cotyledons and of red-brown stained membranes in the intercotyledonary areas. Microscopic changes were minimal and lesions if present, were masked by autolysis.

Included in Text-fig. 3 are the results of the plasma progesterone levels of four cows infected with *V. fetus* in the 7th and 8th months of gestation, and a cow inoculated with sterile water which completed a normal gestation 292 days
after breeding. Pre-inoculation progesterone levels in these cows ranged from 5 to 12 μg/ml of plasma. In general, the progesterone content of the plasma of the cows infected with *V. fetus* remained elevated for 1 to 7 days after inoculation, at which time levels decreased to about 4 μg the last few days.

**Text-fig. 1.** Diagram illustrates the time of gestation when cows were infected with *Vibrio fetus* and the period which elapsed between inoculation and abortion. (H—Hereford, F—Holstein-Friesian, A—Angus.)

**Text-fig. 2.** Concentrations of jugular plasma progesterone from cows in the second 3 months of pregnancy. Arrows under the abscissa indicate the time of abortion following inoculation. (■) 435, inoculated with *V. fetus* Day 122, aborted Day 129. (□) 026, inoculated with *V. fetus* Day 151, aborted Day 157. (△) 29, inoculated with *V. fetus* Day 182, aborted Day 187. (○) 433, inoculated with sterile water Day 188.
before abortion. Progesterone levels of these cows declined to less than 1 μg/ml of plasma about 24 hr before abortion. All aborted foetuses, except that from No. 011, were alive at birth. The foetus from No. 011 was aborted during the night and was decomposed when recovered the following morning. The aborted foetuses from No. 001 and No. 433 died within 30 min after expulsion, while the calf from No. 475 appeared clinically healthy, even though organisms were recovered from the viscera. Placental lesions consisted of multiple, 3- to 5-mm or larger, yellow-grey foci of necrosis in the cotyledons. The thick, oedematous intercotyledonary areas contained numerous small, yellow-grey foci which coalesced in some instances to form large plaques on the chorionic surfaces of the chorio-allantois. Occasionally crater-like lesions with irregular edges were present adjacent to the cotyledons and in the intercotyledonary areas. Microscopic changes consisted of necrotic villi in the cotyledons, necrotic detritus on the chorionic surfaces and ulcerated lesions with masses of fibrin, neutrophils and placental macrophages in the oedematous mesenchyme of the intercotyledonary areas. A common feature, vasculitis, was best developed in medium-sized arterioles.

**DISCUSSION**

The source of plasma progesterone in cows during the second and third 3-monthly periods of gestation is as yet not fully resolved. A number of reports (McDonald, McNutt & Nichols, 1953; Estergreen, Frost, Gomes,
Erb & Bullard, 1967; Erb, Gomes, Randel, Estergreen & Frost, 1968) indicate that the corpus luteum (CL) serves as a principal source of circulating progesterone during the first 200 days of gestation and again from 250 days of gestation to term. Apparently extra-ovarian sites such as the placenta (McDonald et al., 1953; Haterius, 1936) or adrenal (Balfour, Comline & Short, 1957) act as the principal sources of progesterone production from 200 to 250 days of gestation. Although a number of investigators attempted unsuccessfully to recover progesterone from cow placental extracts (Gorski, Erb, Dickson & Butler, 1958; Short, 1957; Bowerman & Melampy, 1962), Ainsworth & Ryan (1967) recently demonstrated that bovine placental preparations are capable of producing progesterone. Even though the sources of progestins during pregnancy are not established in this study, V. fetus appears to have an adverse effect on the foetal placental unit which precedes the decline in plasma progesterone levels.

The rather rapid decline in progesterone levels of cows infected during the 4th, 5th and 6th months of gestation is probably the result of foetal death. Progesterone levels in cattle infected with V. fetus (second 3 months of pregnancy) declined 2 to 4 days before abortion or at the approximate time of foetal death. Although the reason for the decline in progesterone at this time is unknown, a number of possibilities exists. The death of the foetus may remove the placenta as a source of progesterone, or products released from autolysis of the infected foetus and/or placenta may inhibit CL function as it relates to progesterone production. The possibility that V. fetus acts as a luteolytic factor seems unlikely as Coudert & Short (1966) demonstrated that V. fetus prolonged the functional life of the CL in sheep.

The four foetuses infected during the 7th and 8th months of gestation resisted V. fetus more effectively and remained in utero considerably longer than those inoculated earlier; however, all foetuses were expelled before term. Three of the four foetuses were alive at parturition. Placentitis appeared as a common feature. Lesions of this nature may affect the ability of this organ to produce progesterone, thus leading to a premature decline in production of this hormone.

During the last 3 months of pregnancy, plasma progesterone content dropped about 4 mµg several days prior to abortion and then to less than 1 mµg/ml about 24 hr before abortion. The decline in progesterone levels associated with abortions in the last 3 months of pregnancy closely resembles the observations of Short (1958) and the authors (Text-fig. 3) concerning normal parturition in cattle. This suggests that a decline in progesterone production is a necessary prerequisite for foetal expulsion (relaxation of pelvic ligaments, cervix, etc.) in either abortion occurring late in gestation or normal parturition. The results in this study differ from those reported by Short, Wagner, Fuchs & Fuchs (1965), who observed an insignificant decline in progesterone levels of ovarian venous and peripheral plasma progesterone of women given intra-amniotic injections of hypertonic saline to induce abortion. However, their findings in the saline-treated women did not differ from those observed at parturition in normal women where uterine activity increases in spite of high levels of circulating progesterone.
Although abortion is commonly associated with various infectious agents, few reports encompass the microbial, physiological and pathological aspects of this phenomenon. Most workers (Wagener, Bisping & Schulz, 1963; McKay & Wong, 1963; Urbaschek, 1964; Osborne & Smibert, 1964; Dennis, 1966) emphasized the importance of endotoxins or hypersensitivity as the principal mechanisms associated with microbial abortion. The dramatic fall in progesterone levels frequently observed in these cows 1 to 3 days before expulsion suggests a definite association between the plasma progesterone content and abortion. During the second 3 months of pregnancy the progesterone decline is closely correlated with death of the foetus; in the third 3 months of pregnancy the progesterone decline (to 1 µg/ml plasma) heralds the abortion of a live foetus. Although the exact manner in which V. fetus exerts its effects is still not clear, it is possible that V. fetus, or its toxins, affects the production of progesterone through placental dysfunction or foetal death to the extent that the progesterone 'block' of Csapo (1956) is no longer effective in maintaining pregnancy.

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REFERENCES


Progesterone levels in bovine vibrionic abortion


