

FAILURE OF SHEEP \times GOAT HYBRID FOETUSES TO SURVIVE IN GOATS TREATED WITH SHEEP'S BLOOD

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Summary

The survival of sheep \times goat hybrid foetuses in goats was adversely affected by twice-weekly intramuscular injections of 15 ml of sheep's blood into the mother. This procedure had been reported by others to result in the birth of living hybrids. High titres of anti-sheep haemolysins appeared in the maternal serum soon after the injection of blood, and it is suggested that these antibodies hastened foetal death. The maintenance of a low level of passive immunity in the maternal goat by injection of antisera appeared to delay foetal death.

I. INTRODUCTION

Bratanov and Dikov (1961) reported the birth of sheep \times goat and goat \times sheep hybrids from parents that had both been subjected to intramuscular injections, twice weekly, of 10–15 ml of whole blood from the other species; the injections began about 3 months before mating and continued throughout pregnancy. Out of 172 treated sheep and goats, 31 hybrids were obtained. Other workers (Warwick, Berry, and Horlacher 1934; Quinlan *et al.* 1941; Hancock 1964; Alexander, Williams, and Bailey 1967) consistently failed to produce viable hybrids when untreated sheep (*Ovis aries*) were mated with untreated goats (*Capra hircus*); female goats readily conceived when inseminated with sheep semen, but the foetuses rarely survived beyond the second month of gestation.

The cause of death of the hybrid foetuses has not been clearly established but, on the basis of the appearance of anti-sheep haemolysins in the blood of goats bearing hybrid foetuses, Alexander, Williams, and Bailey (1967) advanced the hypothesis that death was due to haemolytic disease. They suggested that red cells of the hybrid entered the maternal circulation and stimulated production of anti-red-cell antibodies which, in turn, entered the foetal circulation. The success of Bratanov and Dikov (1961) might be explained, therefore, by phenomena such as immunological paralysis (see Raffel 1956). The present report deals with an attempt to use the blood-injection method of Bratanov and Dikov (1961) and, at the same time, to examine the immunologic response in goats injected with sheep's blood. A report is also presented on an attempt to suppress production of anti-sheep haemolysins in pregnant goats by treating them with small quantities of these antibodies to destroy antigenic foetal red cells that enter the maternal circulation. A similar method of suppression has been successful in preventing Rh isoimmunization in man (Finn and Clarke 1966; Freda, Gorman, and Pollack 1966).

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II. MATERIALS AND METHODS

(a) Animals and Mating Procedure

Twenty-three of the 30 female goats used were of the Saanen breed and the remaining animals were of miscellaneous breeding. Most were mature animals of unknown age.

A Suffolk, a Dorset Down, an English Leicester, and a Merino ram were used for mating, to provide a variety of genotypes. The Suffolk mated readily with goats, the Dorset Down less readily. The other two would not mate and artificial insemination was used: 1 ml of ram semen, obtained by electro-ejaculation, was flooded over the cervical os. The mating procedure and the method of detection of oestrus were as described by Alexander, Williams, and Bailey (1967).

(b) Intramuscular Injections of Blood

Twelve goats were divided into four groups, each of which was treated with blood from a different ram. The blood, containing trisodium citrate (5 mg/ml of blood) as an anticoagulant, was injected into the muscle of a leg. Clean, non-sterile procedures were used initially, but aseptic methods were adopted after several local infections were encountered; these were treated with penicillin and streptomycin. Injections of blood were given twice weekly, commencing in early February, 2-3 months before the start of the breeding season, and continued until the outcome of matings had been determined, usually at the end of the breeding season, after 5-6 months of treatment. Prior to mid-June, goats in oestrus were mated with the respective donor rams but thereafter only the Suffolk was used. The rams were not injected with goat's blood.

Sera were collected from the goats, usually at weekly intervals until early October, and thereafter at longer intervals in selected animals.

Laparotomy was performed, about 60 days after mating, in goats not observed in oestrus in the meantime.

(c) Intravenous Injections of Blood

To provide a comparison with the antibody response of goats injected intramuscularly, an additional goat was allotted to each of the above groups. The four additional goats received ram's citrated blood (1 ml) twice weekly by intravenous injection; otherwise, treatments were as above.

(d) Antibody Injections

Fourteen goats were mated with the Suffolk ram, and the twelve that were not observed in oestrus within 26-30 days were each allotted to one of five treatment groups. One group (three goats) received no treatment and the others (two or three goats per group) were placed on courses of intravenous injections of sera containing high titres of anti-sheep haemolysins. Injections commenced between the 26th and 30th days after mating, and sufficient serum was given initially to produce calculated titres (see below) of 2, 1, 0.5, and 0.25, assuming that the serum was diluted in fluids corresponding to 12% of body weight as indicated in preliminary experiments on three male goats. Maintenance doses equal to half the initial doses were then given twice weekly. Injections continued until the outcome of pregnancy was indicated by re-appearance of oestrus or by laparotomy findings as above.

The donor sera were collected from 11 of the 12 goats injected intramuscularly (above); the sera were stored frozen, and injected in volumes of 1.4-24 ml.

Sera from the goats in the five groups were collected monthly prior to mating, thereafter weekly to the start of injection, and then twice weekly until the outcome of pregnancy was determined as above.

(e) Determination of Haemolysin Titre in Serum

The titre of haemolytic antibodies against type R and type O sheep red blood cells (see Rendel 1957), in the presence of guinea-pig complement, was determined as described by Alexander, Williams, and Bailey (1967). Titres are expressed as the reciprocal of the highest dilution at which haemolysis was readily detected. Twofold serial dilutions were used for convenience.

III. RESULTS

(a) *Outcome of Mating*(i) *Intramuscular Injections of Blood*

Nineteen matings involving 9 of the 12 goats were carried out (Table 1); one of the other three failed to come into oestrus, apparently through immaturity, one died from undetermined causes, and one from infection (*Proteus* spp.) at the site of injection. At laparotomy about 60 days after mating, foetal remains [twins, age > 53

TABLE 1

DISTRIBUTION OF NORMAL AND LONGER THAN NORMAL OESTROUS CYCLES ACCORDING TO TREATMENT

Length of Oestrous Cycle	No. of Cycles in Goats receiving Injections of:			
	Sheep's Blood, Intramuscularly	Sheep's Blood, Intravenously	Antiserum to Sheep's Blood	No Antiserum
Before mating period				
≤ 24 days	9	2	13	6
> 24 days	4	1	3	—
During mating period*				
≤ 24 days	2	1	3	1
> 24 days	—	—	1	—
Following mating with ram				
≤ 24 days	5	1	8	1
> 24-c. 60 days	5	—	1	—
> 60 days†	9	2	8	3

* Prior to mating with ram.

† Oestrus did not reappear before laparotomy was performed at c. 60 days after mating with ram.

days, judging from foetal length and the chart of Cloete (1939) for ovine foetuses] were found in only one goat (a Saanen mated with the Dorset Down ram); the foetuses appeared to have died shortly before laparotomy 60 days after mating (Table 2). At laparotomy, there was no evidence of pregnancy in the other eight goats that had failed to come into oestrus during the 8 or 9 weeks that followed mating (Table 2). Four of these eight goats mated in August, at the end of the breeding season, and their failure to show oestrus again could have been due to the onset of anoestrus rather than foetal death. Conception may have occurred in the remaining four.

Three of the eight goats that showed no evidence of pregnancy at laparotomy had been mated two or three times and each may have experienced one or two previous hybrid pregnancies (five in all), as indicated by failure of oestrus to reappear within periods ranging from 27–58 days. However, following mating, the incidence of oestrous cycles longer than the normal interval of 12–24 days (Asdell 1964) was only slightly greater than that in cycles that did not follow mating (5 of 10 and 4 of 15 cycles, respectively—Table 1). This suggests that few of the long cycles were due to conception. Long cycles also occurred in untreated does that later received antisera (3 of 16 cycles—Table 1).

(ii) *Intravenous Injections of Blood*

Only two of the four goats were mated. One of the others died from anaphylactic shock following an injection of blood and one failed to exhibit oestrus. One Saanen

showed evidence of abortion, by a bloody vaginal discharge, 46 days after mating with the Suffolk ram. Another came into oestrus 19 days after mating with a Dorset Down ram, but conceived when mated to the Suffolk 20 days later; foetal remains were found at laparotomy 66 days after this mating (Table 2).

TABLE 2

LAPAROTOMY FINDINGS AFTER MATINGS WHICH WERE FOLLOWED BY LONGER THAN NORMAL OESTROUS CYCLES

Matings followed by normal oestrous cycles are shown in Table 1

Injections given to Goats	Total No. of Matings	No. of Goats with:				
		No Evidence of Pregnancy	Uterus Slightly Enlarged	Remnants of Conceptus	Recently Dead Foetus(es)	Apparently Healthy Foetus
Sheep's blood, intramuscularly	14*	8			1	
Sheep's blood, intravenously	2†				1	
Antisera to produce calculated titres of:						
2	2			1		1
1	2		1	1		
0.5	3‡	1				1
0.25	2					2
Controls (no antisera)	3	1		1	1	

* No laparotomy performed in five of these goats because oestrus reappeared before operation was due (laparotomy usually performed about 60 days after mating).

† In one of these goats there was external evidence of abortion and oestrus reappeared before laparotomy was due.

‡ No laparotomy performed in one of these goats because oestrus reappeared before operation was due.

(iii) Antibody Injections

In contrast with the goats injected intramuscularly with sheep's blood, seven of nine that received antiserum showed evidence of pregnancy at laparotomy (Table 2) and, in four of these, the healthy appearance of the enlarged uterus 59–63 days after mating indicated that the animal was still pregnant. When examined 3 weeks later, the uterus was involuting in two animals. The other two still appeared to be pregnant at a further examination at 100 days after mating, but 6 weeks later, debris could be seen floating within the still enlarged and turgid uterus. Mummified foetuses of lengths corresponding with gestation ages of 45 and 60 days were recovered. The incidence and course of pregnancy appeared unrelated to the dose of antiserum (Table 2).

Under this treatment, the incidence of apparently healthy uteri, at about 60 days after mating (4 cases in 8 animals), was significantly higher ($P < 0.02$) than in the untreated goats (2 cases amongst 25 animals) examined by Alexander, Williams, and Bailey (1967) and higher than in the untreated goats in the present study (no cases amongst three animals—Table 1).

(b) Haemolytic Titres

(i) Intramuscular Injections of Blood

Prior to injection, sera of all animals failed to haemolyse sheep *R* or *O* red cells. However, within a week of the first injection, titres of about 32 were found (examples

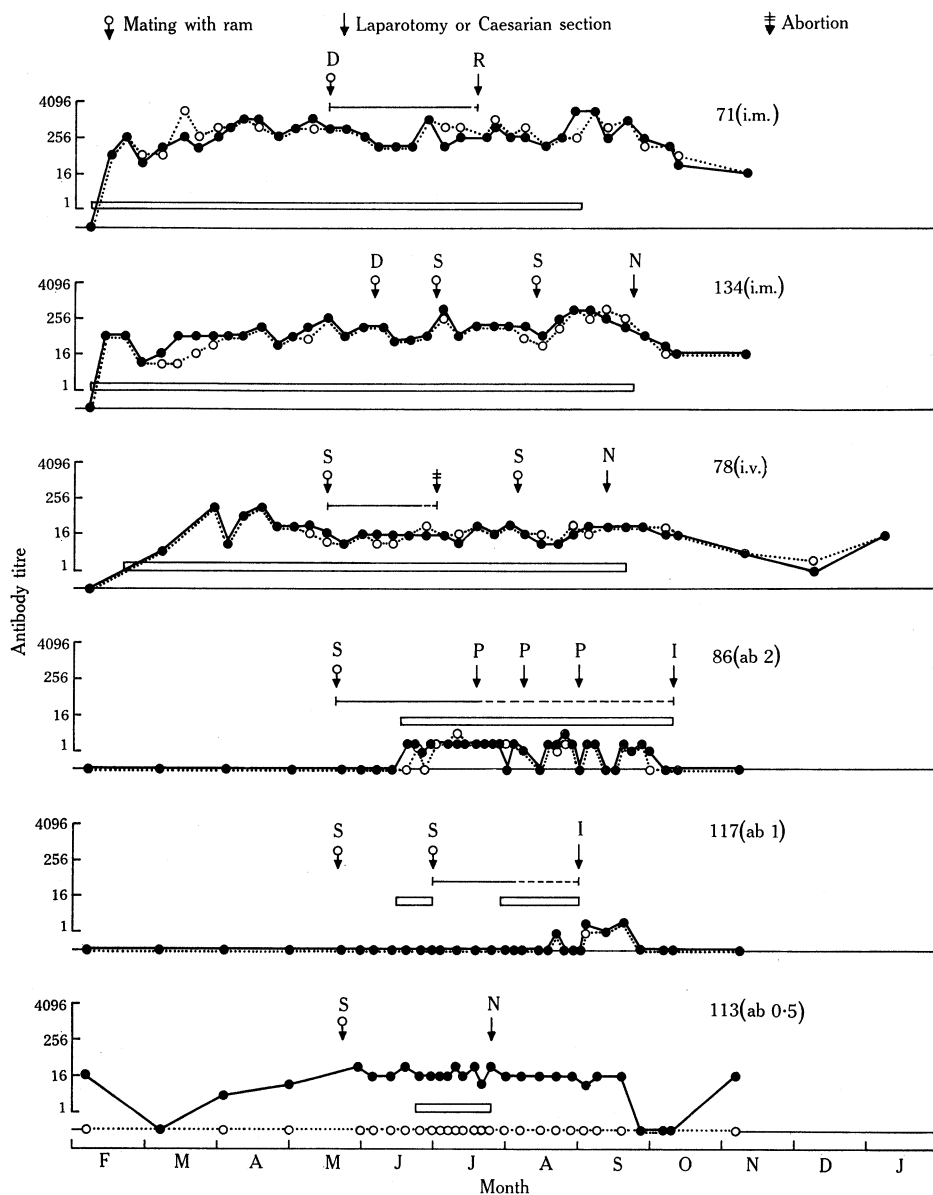


Fig. 1.—Titres of anti-sheep haemolysin in sera of representative goats. Titres are plotted on a logarithmic scale; points shown below a titre of 1 indicate absence of detectable antibody. Anti-O titres are shown by the open circles, anti-R titres by the closed circles. All goats were Saanens; S, Suffolk ram; D, Dorset Down ram. Findings at laparotomy are indicated by: P, uterus appeared healthy and pregnant; R, recently dead foetus; I, uterus involuting; N, uterus completely involuted. The horizontal lines represent the period from mating to laparotomy, the breaks in these lines indicating the apparent age of the foetus at death. Horizontal bars indicate the period of treatment. The treatment is indicated beside the identification number of the goat: i.m., intramuscular injections of sheep's blood; i.v., intravenous injections of sheep's blood; ab, antisera injected to produce calculated titres of 2, 1, or 0.5.

are given in Fig. 1). Titres tended to increase during the following 2 months to reach levels of up to 2048 (usually 256), and high levels were maintained with some appreciable fluctuations (Fig. 1). Titres declined slowly after cessation of treatment. In general, titres against sheep *R* and sheep *O* cells were similar.

(ii) *Intravenous Injections of Blood*

Titres increased rapidly after the start of treatment (Fig. 1) but, in general, were lower and more varied than those in goats receiving blood intramuscularly.

(iii) *Antibody Injections*

Apart from an anti-*R* titre of 16, detected in one goat before treatment (goat 113, Fig. 1), no titre was detected in goats given the lower two doses of antiserum, although pregnancy in three was apparently maintained for more than 2 months (Table 2).

In one of the two goats that received the highest dose, titres of 2 were achieved at the commencement of treatment (goat 86, Fig. 1). In the other, a titre of 1 was detected 2–3 weeks before an involuting uterus was found at laparotomy. A similar situation was found in one of two goats receiving the second highest dose (goat 117, Fig. 1) but, in the other animal, in which involution of the uterus was almost complete at laparotomy, no haemolysin was detected.

Haemolysin was not detected in any of the three untreated animals, although two had obviously been pregnant (Table 2).

IV. DISCUSSION

Treatment of the mother goat by the procedure of Bratanov and Dikov (1961) clearly failed to promote survival of the foetal hybrids in the limited number of goats used in the present study. On the contrary, the evidence suggests that foetal death usually occurred earlier in pregnancy (Table 2) than was observed in untreated animals (Alexander, Williams, and Bailey 1967; present study). Presumably, in the twin foetuses that survived for about 2 months, the barrier between the maternal and foetal circulations was not disrupted until relatively late. There was no evidence that the immune response to intramuscular injections of sheep's blood differed qualitatively from that after intravenous injection. The high titres attained and the early foetal death are consistent with our hypothesis (Alexander, Williams, and Bailey 1967) that foetal death results when anti-sheep haemolysin reaches the foetus from the maternal circulation. However, less specific adverse effects of such an extreme procedure as repeated and massive blood injections cannot be completely discounted as a cause of pregnancy failure.

In contrast, the production of a low level of passive immunity in pregnant goats appeared to promote survival, although the age at which foetal death occurred is uncertain, since in two animals the uterus appeared normal and gravid long after foetal death had occurred. There is little doubt, however, that because of retarded growth and post-mortem shrinkage, the two foetuses recovered 140 days after mating were older than their lengths indicated (45 and 60 days). The preservation of the gravid appearance of the uterus long after foetal death is unusual and may be associated with the antibody treatment.

In prevention of Rh isoimmunization in man (Finn and Clarke 1966; Freda, Gorman, and Pollack 1966), treatment is confined to the period immediately after birth, since appreciable quantities of foetal blood enter the maternal circulation during parturition only (Woodrow and Finn 1966). In the present study, the apparent need for continued treatment and the possibility of foetal damage by the treatment no doubt greatly reduced the chances of success.

The appearance of anti-red-cell antibodies in the serum of two animals at about the time of presumptive foetal death (example in Fig. 1) is consistent with our previous report (Alexander, Williams, and Bailey 1967) and suggests that a low level of passive immunity may be insufficient in some pregnancies to remove all foetal red cells from the maternal circulation. However, the failure of the untreated pregnant goats to develop detectable levels of antibody contrasts with the consistent appearance of antibody in the previous study. This need not invalidate the hypothesis that foetal death in the hybrids is caused by anti-sheep haemolysins; foetal death could result from antibody levels below the limit of detection by the present method.

While the results of this study can be explained by our hypothesis, proof of its validity is yet to be obtained and the success of Bratanov and Dikov (1961) presumably lies in the use of sheep and goats with immunologic responses different from those of our animals.

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