THE EFFECT OF AMYLASE, CATALASE, AND A DECAPACITATING PREPARATION ON FERTILITY OF BULL SEMEN DILUTED IN AMBIENT TEMPERATURE EXTENDER

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Summary

Four ejaculates from each of three bulls were diluted in four ambient temperature extenders: (1) Caprogen; (2) Caprogen containing 8 μg/ml α-amylase; (3) Caprogen containing 4·5 μg/ml catalase; and (4) Caprogen containing amylase and catalase. The conception rate (49-day % non-return rate) for each extender was 64·63; 63·37; 64·58; and 66·09% respectively. Whereas the addition of catalase significantly increased conception rates ($P = 0{\cdot}025$), the addition of amylase did not ($P > 0{\cdot}25$). There was a significant enzyme interaction, suggesting that any beneficial effect attributable to the amylolytic preparation was only exerted in the presence of catalase ($P < 0{\cdot}025$). The significant improvement in *in vitro* livability at 37°C by the addition of amylase in the absence of catalase was minor compared to the improvement in livability obtained by the addition of catalase.

In a second experiment another 12 ejaculates were each diluted in two extenders—Caprogen plus catalase, and Caprogen plus catalase plus 5% of a semen dialysate. This solution was presumed to contain some of the low molecular weight decapacitation factor found in bull semen. Average conception rates for the two extenders were 64·87 and 66·32% ($P = 0{\cdot}12$) and *in vitro* livabilities were 113·5 and 110·5 hr respectively ($P > 0{\cdot}25$).

I. INTRODUCTION

The optimum time to artificially inseminate dairy cattle is from mid-oestrus to the end of standing oestrus, with only slightly lowered conception rates resulting from insemination within the next 6 hr (Salisbury and VanDemark 1961). Since some sperm may be found close to the site of fertilization within minutes of cervical insemination (VanDemark and Moeller 1950, 1951), but ovulation does not occur until an average of 12 hr after the end of standing oestrus, it is presumed that the decline in conception rate associated with post-oestral insemination is the result of insufficient time for capacitation to be completed. Although Mahajan and Menge (1966) could not demonstrate the necessity for capacitation in dairy cattle, the presence of a potent decapacitation factor (DF) of low molecular weight in bull semen (Williams *et al.* 1967) does suggest that, even though the process may be rapid, bull sperm must be capacitated.

After Williams *et al.* (1967) reported that β-amylase, or a contaminant in their preparation, inactivated rabbit seminal plasma DF, Kirton and Hafs (1965) showed that rabbit sperm could at least be partially capacitated *in vitro* by incubation in

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β-amylase preparations. The sequel to this study was to demonstrate that when either α- or β-amylase was added to yolk–citrate–glycerol extender used in diluting bull semen, there was a significant increase in conception rates (Kirton, Boyd, and Hafs 1968). The average increase of 2·6% was considered to be approximately equal to the decline in overall conception rates resulting from post-oestral inseminations.

The following experiments were designed to test whether similar improvements in conception rates could be obtained by the addition of amylase to ambient temperature extender, and if the dialysable fraction of bull semen, which was presumed to contain DF, influenced conception rates.

II. Materials and Methods

In the first experiment, four ejaculates from each of three Jersey bulls were diluted to a final concentration of between 4·7 and 5·0 million total sperm per millilitre in Caprogen (Shannon 1965*b*). Each diluted ejaculate was divided into four 500-ml portions containing: (1) no added enzymes; (2) 8 μg/ml of α-amylase (Sigma Type II-A from *Bacillus subtilus*); (3) amylase plus 4·5 μg/ml of catalase prepared from bull's liver; and (4) catalase alone. All the inseminations, of 0·5 ml, were made either 27–30 hr after collection (two bulls) or 49–52 hr after collection (one bull) in districts organized by the Taranaki Herd Improvement Association.

In the second experiment, four ejaculates from each of the same three bulls were used. The two extenders compared were Caprogen plus catalase, and Caprogen plus catalase plus 5% of a semen dialysate. This dialysate was prepared by dialysing bull semen which had been stored at −79°C immediately after collection, against double its thawed volume of 2% sodium citrate. Fructose was measured before and after dialysis as an indicator of the proportion of low molecular weight particles which had been dialysed.

Prior to dispatching the extended semen, a 5-ml aliquot from each batch was incubated at 37°C so that *in vitro* livability could be assessed (Shannon 1965*b*).

Statistical analyses were conducted on the conception rates (49-day % non-return rate to first insemination). The variance was partitioned between bulls, treatments, the interaction between bulls and treatments, ejaculates within bulls, ejaculates within bulls within treatments (which included random error), and the binomial error term similar to that used by Shannon (1965*a*). In the first experiment, the treatment variation was partitioned between amylase *v.* no amylase, catalase *v.* no catalase, and the interaction between the two enzymes.

III. Results

The average number of first inseminations obtained from each of the 48 batches in the first experiment was 568±19 (S.E.). Differences between bulls in conception rate (65·92, 66·64, and 61·46%) were statistically significant ($P < 0·01$), but neither the bull by treatment interaction nor the interaction of batches of semen, confounded within bulls within treatments (plus random error), was significant ($P > 0·25$). The ejaculate within bull interaction was significant ($P = 0·001$).

The differences between the averages for the four treatments were significant ($P < 0·01$), being attributable to a significant improvement through the use of catalase (65·34 *v.* 64·00%; $P = 0·025$), no demonstrable effect through the use of amylase (64·61 *v.* 64·73%), and a significant interaction resulting from the use of the two enzymes ($P = 0·025$). Reference to Table 1 shows that, whereas the addition of amylase alone resulted in the lowest conception rate (63·37%), when it was used in combination with catalase the highest conception rate was obtained (66·09%).

The addition of catalase to the extender resulted in a significant increase in *in vitro* livability (103·5 *v.* 71·7 hr; $P < 0·001$). In contrast to the conception rate

data, the addition of amylase increased *in vitro* livability in the absence of catalase (74·8 *v.* 68·3 hr; $P < 0{\cdot}05$). Neither differences between bulls nor the bull by treatment interaction was significant ($P > 0{\cdot}25$). The ejaculate within bull interaction was significant ($P < 0{\cdot}01$).

The fructose assay of the semen before and after dialysis showed that 72% of this low molecular weight indicator had passed through the dialysis membrane. The extender which contained 5% of the dialysate had a concentration of fructose which was 3·6% of that originally measured in the semen used for dialysis. In the second experiment, the average number of first inseminations for each of the 24 batches was 479±109 (S.E.). As with the first experiment, differences between bulls and ejaculates within bulls were statistically significant ($P < 0{\cdot}05$). However, the addition, to extenders containing catalase, of DF (assumed present in semen dialysate) did not affect either conception rate [64·87% without DF (6796 inseminations) *v.* 66·32% with DF (4689 inseminations); $P = 0{\cdot}12$] or *in vitro* livability (113·5 hr without DF *v.* 110·5 hr with DF; $P > 0{\cdot}25$).

TABLE 1

CONCEPTION RATES AND *IN VITRO* LIVABILITIES FROM EXTENDERS CONTAINING AMYLASE AND CATALASE

	Catalase and Amylase Absent	Catalase Absent, Amylase Present	Catalase Present, Amylase Absent	Catalase and Amylase Present
Conception rate (%)*	64·63	63·37	64·58	66·09
No. of inseminations	6717	6365	7794	6375
Livability (hr)†	68·3	74·8	104·8	102·2

* 49-day % non-return rate to first insemination. † *In vitro* at 37°C.

IV. DISCUSSION

Although Dukelow, Chernoff, and Williams (1966) and Williams *et al.* (1967) reported that β-amylase inactivated rabbit DF, the former subsequently found that when using a highly purified β-amylolytic preparation they could not demonstrate *in vitro* capacitation as was reported by Kirton and Hafs (1965). Since β-amylase is rarely found in mammals (see White, Handler, and Smith 1964), it is unlikely that this enzyme is essential for *in utero* capacitation. Murdoch and White (1968*a*) detected significant levels of α-amylase in rabbit semen but only low cencentrations in bull semen. They concluded that it appeared unlikely that α-amylase was the capacitation factor.

Nonetheless, Kirton, Boyd, and Hafs (1968) and Hafs *et al.* (1969) have reported separate trials, both of which showed that crude preparations of α-amylase (50–100 units/mg), when added to extenders for bull semen at 10 μg/ml significantly increased conception rates. The former trial also showed that the addition of β-amylase increased conception rates. Since both emzymes tended to enhance *in vitro* livability at 5°C, Kirton, Boyd, and Hafs (1968) suggested that amylase may have affected sperm capacitation or that amylase may beneficially influence substrate availability for sperm metabolism. The results of the present experiment showed that the addition

of 8 μg of α-amylase significantly increased *in vitro* livability at 37°C, although Murdoch and White (1968*b*) found that this enzyme did not increase metabolic rate.

Because of the likelihood that impurities present in the amylolytic preparations originally used by Dukelow, Chernoff, and Williams (1966), Kirton and Hafs (1965), and Kirton, Boyd, and Hafs (1968) could have influenced their results, highly purified α-amylase (500–1000 units/mg) was used with the Caprogen. Consequently, the level of 8 μg/ml of extender would represent a concentration at least eight times greater than the higher level used by Kirton, Boyd, and Hafs (1968). Whereas the Caprogen would have contained 4–8 units/ml of α-amylase, the normal value for bull semen is only 0·022 units/ml (Murdoch and White 1968*a*). With this high level of α-amylase the lowest conception rate was obtained. This reduction in conception rate when amylase was added to Caprogen occurred in spite of slightly improved *in vitro* livability. Hafs *et al.* (1969) also found that when α-amylase was added at 5–10 units/ml of extender, conception rates were depressed.

There appeared to be a beneficial synergism between amylase and catalase, as the addition of both enzymes to Caprogen significantly increased conception rate. A speculative explanation for this effect is that these high levels of amylase may accelerate the metabolic change *in utero* which results in increased oxygen utilization (Hamner and Williams 1962; Dukelow, Chernoff, and Williams 1967; Murdoch and White 1967; Soupart, 1967). Shannon (1965*b*, 1968) has shown that the saturation of extender with nitrogen and the addition of 4·5 μg/ml catalase are beneficial in minimizing the effect of toxic end products of oxygen utilization on sperm livability. The addition of catalase together with a high level of amylase may allow the beneficial effect of the latter enzyme to be exerted. The results for livability clearly demonstrate the beneficial effect of catalase on *in vitro* storage at 37°C.

In contrast to the previous results obtained by Shannon (1968, 1969), the addition of catalase alone did not increase conception rate. However, a detailed analysis of his results showed that the improvement obtained through using catalase in Taranaki was only 0·8%, which was lower than elsewhere in New Zealand.

Although considerable endeavour has centred on the approach that capacitation is a barrier to fertility, scant attention has been paid to the possibility that there is a physiological explanation as to why sperm are uncapacitated prior to entry into the uterus. Bull semen is most suitable for the study of this latter aspect because a significant fraction of the DF is a material with a molecular weight of approximately 500 (Williams *et al.* 1967) and should therefore be readily dialysable. Although DF may not be identical to the compound which may be removed from the sperm in the process of capacitation, it rapidly alters capacitated sperm to a state where they must be recapacitated. Since the addition of DF to the uterus also prevents or alters the capacitation sequence (Williams *et al.* 1967), a DF preparation should influence fertility if the capacitation process is limiting the attainment of optimal conception rates.

If the low molecular weight fraction of the DF in bull semen were as dialysable as fructose, the experimental extender contained dialysable DF at a concentration equivalent to about 3% of that found in the original semen. Since the experimental semen was diluted at about 1 ml semen to 300 ml of extender, the concentration of the DF per sperm would be high. The results in Section III show that the addition of the

semen dialysate did not compromise fertility and may have been beneficial, as the conception rate of 66·32% was equal to that obtained by the addition of both catalase and amylase.

If, in fact, the semen dialysate contained a significant concentration of DF, the results suggest that the presence of seminal plasma DF does not compromise the capacitation sequence in dairy cattle and that any beneficial effects attributable to the addition of amylase to semen extenders are not mediated through the removal or inactivation of seminal plasma DF. Since completion of these experiments, Shannon (personal communication) has demonstrated that bull semen contains a dialysable metabolic inhibitor or regulator which reduces oxygen utilization at 15–20°C. It may not be operative at 37°C when glycolysis is not inhibited (Blackshaw, Salisbury, and VanDemark 1957). Therefore, any beneficial effect exerted by the addition of the dialysable fraction of seminal plasma to extender may also be through improved storage prior to insemination.

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