

# Fertilization and embryonic mortality rates in beef heifers after artificial insemination

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**Summary.** A total of 256 beef heifers, in 2 experiments, was used to establish fertilization rate and subsequent embryo survival rates. Fertilization rate following a single artificial insemination was 90%. Pooled estimates of embryo survival showed high survival rates up to Day 8 (93%) but markedly reduced ( $P < 0.001$ ) survival at Days 12 (56%), 16 (66%) and 42 (58%). It is suggested that most embryonic mortality occurs between Days 8 and 16.

## Introduction

Reproductive failure is one of the major factors affecting output in beef and dairy herds. While it is generally accepted that fertilization rates after natural or artificial insemination are normally close to 90%, calving rates to a single insemination are closer to 50%. Fertilization rates of 96–100% have been reported following the use of bulls of proven fertility (Laing, 1949; Kidder, Black, Wiltbank, Ulberg & Casida, 1954; Bearden, Hansel & Bratton, 1956), but embryo survival rates are considerably lower. Henricks, Lamond, Hill & Dickey (1971) showed a fertilization rate of 89%, but the proportion of embryos surviving at Day 42 after insemination was only 60%. Calving rates to a single insemination are reported to be 50–55% for heifers (Sreenan & Mulvehill, 1975; Roche, Prenderville & Davis, 1977; Wishart, Young & Drew, 1977), 52–57% for dairy cows (Mawhinney & Roche, 1978) and 53% for beef cows (Roche *et al.*, 1977).

While these reports demonstrate the existence and extent of embryonic mortality, few of them indicate the time at which it occurs. It has been suggested that most losses occur before Day 15 after breeding (Ayalon, 1972). For repeat breeder cows Ayalon (1973) has suggested Day 7 as the critical day on which embryonic death occurs.

The aim of the present study was to determine the fertilization and embryo survival rates at various times after a single artificial insemination in beef heifers.

## Materials and Methods

### *Animals*

The 256 sexually mature beef heifers used were Aberdeen Angus and Hereford crosses and weighed 350–425 kg. The animals were purchased directly for these experiments and their previous reproductive histories were unknown. Animals with detectable genital abnormalities were excluded before or during the experiments. Oestrus was induced synchronously in all animals by using a 9-day intravaginal progesterone treatment (Sreenan & Mulvehill, 1975) or a subcutaneous progestagen (Norgestomet; G. D. Searle & Co.) implant (Wishart & Young, 1974). It has been shown that fertilization rate, early embryonic development, and subsequent

pregnancy rates are not affected following the use of short-term progestagen treatments for synchronization of oestrus (Roche, 1974; Wishart & Young, 1974; Sreenan & Mulvehill, 1975). In the present experiments, short-term progestagens were used to facilitate replication of experimental groups.

All animals were checked for oestrus with the aid of vasectomized bulls and were inseminated only on the basis of an observed standing oestrus (Day 0). Inseminations were carried out by the same technician 8–12 h after the first observation of standing oestrus. Frozen–thawed semen (approximately  $30 \times 10^6$  spermatozoa/straw) from one bull of high fertility (90-day non-return rate of 76% based on 25 000 first inseminations) was used throughout the experiments. The heifers were randomly allotted to treatments after insemination.

### Experiments

*Experiment 1.* A total of 119 heifers was used to establish fertilization and embryo survival rates at Days 4, 8, 12 and 42 after insemination.

*Experiment 2.* Arising from the data in Exp. 1 and mainly because of the low embryo survival rate recorded at Day 12, which may have been due in some cases to technical problems, it was decided to repeat the experiment. Fertilization and embryo survival rates at Days 8, 12, 16 and 42 after insemination were determined for 127 heifers.

### Embryo survival

The method of estimation depended on the day of study. Ovum recovery procedures were as follows.

*Day 4.* These animals were laparotomized and the oviduct and uterine horn tip ipsilateral to the corpus luteum were flushed *in vivo*.

*Day 8.* At laparotomy the oviduct and approximately half of the ipsilateral uterine horn were flushed.

*Day 12.* The oviduct and most of the ipsilateral uterine horn were flushed *in vivo* (in half of the animals) or immediately after slaughter in the remainder.

*Day 16.* The entire ipsilateral horn was flushed immediately after slaughter.

*Day 42.* The uteri were checked at slaughter for evidence of viable pregnancies.

*Flushing procedures for embryos in vivo.* Closed-circuit general anaesthesia was used for recovery of embryos *in vivo* (Sreenan & Beehan, 1974). The flushing medium used was phosphate-buffered saline with 10% (v/v) bovine serum albumin. The flushings were examined initially under a stereoscopic microscope to locate the ova, morulae or blastocysts, which were then transferred as fresh mounts to an inverted light microscope for more detailed examination. When there was any doubt about normality of the embryos, they were fixed in acetic alcohol (1:3 v/v) or Bouin's fluid for further examination.

*Fertilization, viability and embryo survival criteria.* Fertilization rate at Day 4 was based on the recovery of cleaved zygotes containing 4–16 blastomeres. Viability at this stage was based on the occurrence of blastomeres of even granulation and nearly equal size which contained nuclei or mitotic figures when fixed and stained. Fertilization rate at Day 8 was based on the recovery of cleaved zygotes, morulae or blastocysts and viability was determined from the recovery of blastocysts with a well formed blastocoele and inner cell mass. At Day 12, viability was based on the recovery of hatched expanded blastocysts with an inner cell mass and typical wrinkled appearance. At Day 16, viability was based on the recovery of elongated blastocysts with an embryonic disc. At Day 42 pregnancy diagnosis was based on the recovery of fetuses and fetal fluids, and viability on expected weight for age of the fetus and fetal fluids, and also on the appearance of the whole conceptus (Sreenan & Beehan, 1976).

Embryo survival was calculated as the number of viable embryos recovered as a proportion

of the number expected. The expected number of embryos was based on the overall fertilization rate recorded for Days 4 and 8 combined within each of the two experiments.

### Statistical analysis

The difference between proportions was examined by  $\chi^2$  tests.

## Results

Of the 256 animals presented for insemination 10 were discarded, 5 because of ovulation failure following the synchronized oestrus, 2 because of follicular cysts, 2 because of severe adhesions between the fimbriae and ovaries and one because of blocked oviducts.

### Experiment 1

The results are given in Table 1. Recovery rates at Days 4 and 8 were similar and were higher than those at Day 12 ( $P < 0.05$ ) and 42 ( $P < 0.025$ ) which were also similar. Recovery rates at Day 12 after recovery *in vivo* and *in vitro* were 61 and 58% respectively ( $P > 0.10$ ). Fertilization rates were similar for Days 4 and 8.

**Table 1.** Recovery, fertilization and embryo survival rates on different days after artificial insemination in beef heifers (Exp. 1)

	Day after insemination			
	4	8	12	42
No. of heifers inseminated	35	18	37	29
No. of heifers with embryos (%)	30 (86) <sup>a</sup>	16 (89) <sup>a</sup>	22 (59) <sup>b</sup>	16 (55) <sup>b</sup>
Fertilization rate (%)	27 (90) <sup>a</sup>	14 (88) <sup>a</sup>	18 (82) <sup>a</sup>	—
No. of viable embryos	27	14	15	15
Embryo survival rate* (%)	100 <sup>a</sup>	100 <sup>a</sup>	45 <sup>b</sup>	58 <sup>b</sup>

\* No. of viable embryos/no. expected (see text).

Within rows, values with different superscripts are significantly different,  $P < 0.05$  at least.

Embryo survival rates were lower at Days 12 and 42 than at Days 4 and 8 ( $P < 0.005$ ) for all comparisons.

Two of the 5 unfertilized ova recovered at Days 4 and 8 had an abnormally large perivitelline space with a small contracted cytoplasmic volume while the remaining 3 were apparently normal unfertilized ova. Three embryos considered retarded were recovered at Day 12. These embryos had hatched but failed to re-expand and had not reached the expected stage of development for a Day-12 embryo. One degenerating fetus was observed at Day 42; it consisted of remnants of embryonic tissue and membranes.

### Experiment 2

The results are given in Table 2. Recovery rate at Day 8 was higher than at Days 12 ( $P < 0.05$ ), 16 ( $P < 0.01$ ) or 42 ( $P < 0.01$ ) which were all similar. Recovery rates at Day 12 were not different after flushing *in vivo* or *in vitro* and were 67 and 78% respectively ( $P > 0.10$ ). Fertilization determined at Day 8 was 92%.

**Table 2.** Recovery, fertilization and embryo survival rates on different days after artificial insemination in beef heifers (Exp. 2)

	Day after insemination			
	8	12	16	42
No. of heifers inseminated	26	18	39	44
No. of heifers with embryos (%)	25 (96) <sup>a</sup>	13 (72) <sup>b</sup>	24 (62) <sup>b</sup>	25 (57) <sup>b</sup>
Fertilization rate (%)	23 (92) <sup>a</sup>	12 (92) <sup>a</sup>	24 (100) <sup>a</sup>	—
No. of viable embryos	20	9	23	23
Embryo survival rate* (%)	(87) <sup>a</sup>	(56) <sup>b</sup>	(66) <sup>b</sup>	(53) <sup>b</sup>

\* No. of viable embryos/no. expected (see text).

Within rows, values with different superscripts are significantly different,  $P < 0.05$  at least.

Embryo survival rates for Days 12, 16 and 42 were all similar but lower than for Day 8 ( $P < 0.05$ ). At Day 8 one of the 2 unfertilized ova recovered was considered abnormal and similar to those recovered at Days 4 and 8 in Exp. 1. Three embryos recovered at Day 8 were considered retarded and incapable of further development: 2 were morulae which showed clear evidence of degeneration while the other was an 8–12-cell embryo. At Day 12, 3 retarded embryos similar to those recovered in Exp. 1 were recovered, while at Day 16 one embryo, showing clear evidence of cellular degeneration and therefore considered retarded, was recovered. Two degenerating fetuses were recorded at Day 42.

### Experiments 1 and 2

The data from the two experiments were pooled. The fertilization rate was consistent for both experiments and the overall proportion of fertilized ova recovered at Days 4 and 8 was 64/71 (90%). The overall estimates of embryo survival were 100, 92, 51, 66 and 58% on Days 4, 8, 12, 16 and 42 respectively. It is clear that most embryonic loss occurs after Day 8.

### Discussion

Published estimates of fertilization are 88–100% after the use of fresh semen or natural service (Laing, 1949; Kidder *et al.*, 1954; Bearden *et al.*, 1956; Henricks *et al.*, 1971; Ayalon, 1972) and 82–95% for frozen–thawed semen (Wishart & Young, 1974; Spitzer, Niswender, Seidel & Wiltbank, 1978; Shelton, Heath, Old & Turnbull, 1979). The present overall fertilization rate of 90% after using frozen–thawed semen is therefore satisfactory. However it is probable that all of these values overestimate those achieved in practice because animals with genital abnormalities have been excluded, as in the present studies in which 10 heifers (4%) were discarded because of definite reproductive abnormalities.

Of the ova that were not fertilized in the 2 experiments, 3 had a very contracted cytoplasm and were probably incapable of being fertilized, as suggested by Kidder *et al.* (1954) and Hancock (1962). Nevertheless a very high proportion of fertilizable ova are shed.

The ovum recovery rate *in vivo* of 86% at Day 4 in Exp. 1 is comparable to the 85% obtained by Wishart & Young (1974) and is slightly higher than the 78% reported by Spitzer *et al.* (1978) for recovery at a similar stage. It is also in good agreement with the reported *in-vitro* recovery rates of 79–99% for this stage (Kidder *et al.*, 1954; Henricks *et al.*, 1971; Ayalon, 1972).

The Day-8 recovery rates of 89 and 96% for Exps 1 and 2 respectively are higher than the 68.8% reported by Ayalon (1972) over the period Days 6–10. There are few other reports in the literature of recovery at this stage. High recovery rates are expected up to and including Day 8

because the eggs are (a) still inside the zona pellucida and are therefore easily recognized, and (b) located within the oviduct or at the tip of the uterine horn close to the uterotubal junction whence they are comparatively easily flushed. Retention of the zona pellucida by all ova whether unfertilized, fertilized, or fertilized and degenerating at this stage means that there is an equal chance of recovering each type, and that failure to recover is of a technical nature and not due to embryonic mortality. At Days 4 and 8, therefore, embryo survival can be calculated as the number of viable embryos as a proportion of all fertilized ova recovered. However, after Day 8 the position in relation to failure to recover an embryo is not as clear. Hatching of the embryo occurs between Days 9 and 10 (Winters, Green & Comstock, 1942; unpublished observations). Before shedding the zona pellucida the blastocyst contracts and after hatching it re-expands. During the period of re-expansion, blastocysts between Days 10 and 12 may vary in size and morphological appearance thus giving rise to some difficulty in blastocyst recognition and assessment of viability. If embryo death occurs soon after hatching the blastocysts will fail to re-expand and will then certainly be unrecognizable at Day 12. Failure to recover embryos at Day 12 may therefore be due partly to technical failure and partly to embryo death. Boyd, Bacsich, Young & McCracken (1969) also reported difficulty working at this stage and suggested Day 14 as a more reliable day. Recovery rate at Day 12 in Exp. 1 was 59% and was significantly lower than at Day 8. While recovery rate at Day 12 in Exp. 2 was higher (72%) than in Exp. 1, it was nevertheless significantly lower at Day 8 within Exp. 2. However, the overall proportions were not different for the in-vivo (63%) and in-vitro (64%) flushing, although repeated flushing was possible *in vitro*. While there is considerable variation in blastocyst length at Day 16 (Hawk, Wiltbank, Kidder & Casida, 1955; Greenstein & Foley, 1958) there is no problem in recovery or recognition of blastocysts that have reached this stage of development, because they have elongated and are 50–80 mm in length.

All fertilized ova recovered at Day 4 had the blastomere number and morphological appearance consistent with viable embryos at this stage. This is similar to the findings of Wishart & Young (1974) who observed normal development of embryos up to Day 4 following either natural heat or an induced heat using a short-term progestagen treatment. The Day-8 embryo survival rates of 100 and 87% respectively for Exps 1 and 2 give a pooled survival rate of 93% at Day 8. Ayalon (1972) reported an embryo survival rate of 83% at Day 6/7 in cows, but there are no other similar experiments reported in the literature.

Apart from the recovery problems noted above, it is clear that some embryo loss occurs between Days 8 and 12, because in each experiment 3 retarded embryos were recovered. It is probable that the actual survival rates at this stage are higher than that obtained in these studies and lie somewhere between the survival rates for Days 8 and 16. The 66% embryo survival rate at Day 16 would indicate that the greater part of embryonic loss has occurred by this time. This is consistent with the findings of Boyd *et al.* (1969), who, while observing a low overall incidence of embryonic mortality, concluded that most fertility losses have occurred by Day 15 after breeding and probably much earlier. However, our results and those of Boyd *et al.* (1969) are in conflict with those of Ayalon (1972) who concluded that significant differences between fertilization rates and embryo survival rates only become evident after Day 16.

At Day 42, embryo survival rates were the same (58%) for both experiments, and an additional embryonic loss of 8% between Days 16 and 42 is indicated. The 3 fetal deaths were estimated to have occurred much later than Day 16 and probably close to Day 30. The overall embryonic loss of 42% between Days 4 and 42 is higher than the earlier estimates of about 20% in heifers (Laing, 1949; Hawk, Tyler & Casida, 1955; Erb & Holtz, 1958). However, the overall pooled embryo survival rate of 58% is consistent with reported calving rates of 50–55% after a single insemination in normal cyclic heifers (Sreenan & Mulvehill, 1975; Roche *et al.*, 1977; Wishart *et al.*, 1977), but is considerably lower than the 90-day non-return rate (76%) for this bull. However, this value was achieved with mature cows, while the beef heifers used in the present study were purchased directly and had unknown reproductive histories. It has also been

observed that heifers are generally of lower fertility than mature cows (Boyd & Reed, 1961). While 90-day non-return rates are a useful indicator of the relative fertility of bulls within a stud they are not an accurate estimate of pregnancy rates, since a proportion of females failing to become pregnant are not re-inseminated for such reasons as failure to detect oestrus, late embryonic mortality, anoestrus, culling and the use of natural service.

This study indicates that in genitally normal heifers, fertilization failure accounts for only about 10% of overall reproductive failure while embryo death accounts for more than 30%. The major portion of this embryo loss would seem to occur between Days 8 and 16.

We thank G. D. Searle & Co. Ltd for the supply of Norgestomet implants, Mr T. McDonagh, Mr D. Morris, Mr G. Morris, Mr G. Burke and Mr P. Creaven for help at all stages of this work, which was supported by the Commission of the European Communities.

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Received 3 December 1979