WHY DO BULLS DIFFER IN FERTILITY?

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SUMMARY

The importance of the rate of embryonic death as a factor contributing to fertility differences between bulls used for artificial breeding is discussed. Examples are presented which highlight the necessity to correct for return intervals of more than 30 days from insemination which may arise through poor oestrus detection. Data from 41 bulls showed that the correlation coefficient between the 49-day non-return rate to first insemination and the percentage of return intervals from all inseminations between 18 and 24 days was — 0.98. The major fertility differences between sires therefore involve factors related to fertilization rate. These differences occurred largely with early and mid-oestrous inseminations when non-return rates for sires with above average fertility were 15% and 6% higher than comparable inseminations for sires with below-average fertility. These results highlight the importance of *in vivo* sperm livability as a major factor influencing a sire's fertility.

The principle objective of every artificial breeding organization is to facilitate the widespread use of sires which are either proven or thought to be genetically superior. The achievement of this objective is partly influenced by the fertility levels of the sires to the extent that "semen quality" commonly refers to factors that may be related to fertility rather than genetic factors related to production parameters. Where there is sales competition between different organizations, the fertility levels expressed as non-return rates or conception rates can be as important as the genetic merit of the available sires. O'Conner (1972) clearly states that "No bull whose fertility in practice falls below the acceptable minimum can be used routinely no matter how good he may be in other respects". The acceptable minimum is not stated but in most organizations it will be an arbitrary assessment of whether the further use of such a sire could compromise future sales.

NON-RETURN RATES AND FERTILITY

Comparing fertility levels is often complicated by the different methods used in calculating non-return rates which are commonly used as an index of fertility. These figures may also be different from final calving rates. Pelissier (1972) found that the true conception rate to first insemination in a large Californian study was only 44.2% whereas most commonly

quoted 60- to 90-day non-return rates were 65% to 75%. Graham (1968) reported that semen extender inadvertently frozen without the sperm subsequently produced a 60- to 90-day non-return rate of 18% which rose to 24% when the extender contained only dead sperm. Some of this confusion obviously arises through assuming that failure to record a re-insemination within the one organization means that a cow is in-calf.

The thoroughness of oestrus detection may also influence results. Casida *et al.* (1946) determined pregnancy by palpation and showed that the 30- to 60-day non-return rate was 15.3% higher than the conception rate. The comparable discrepancies with 60- to 90-day and 90- to 120-day non-return rates were 6.1% and 3.0%, respectively. Such discrepancies may not have been considered by Salisbury (1968) who reported that the embryonic death rate between 30 and 180 days of pregnancy increased from 14.1% with unfrozen semen used the day following processing to 21.4% when used 3 days later, whereas the results for fertility at 30 days after insemination were 80.3% and 60.8%, respectively.

THE EFFECT OF OESTRUS DETECTION

The influence of not detecting all cows which fail to conceive when they are next in oestrus about 3 weeks later can be seen in the following example. If 20% of the cows which fail to conceive are next recorded in oestrus about 6 weeks following insemination, then 4% of the cows will record 9-week return intervals and another 0.8% will have 12-week intervals. This means that 25% of all return intervals will be greater than 30 days merely because of failures in oestrus detection. This proportional relationship should be constant for that population of cows which fails to conceive, and consequently every 4% decline in 30-day non-return rate will be associated with a 1% increase in return intervals of more than 30 days. It is unlikely to be a random error which is self-cancelling as suggested by Salisbury and Flerchinger (1967).

The effect of varying the incidence of multiple cycling due to missed heats on the estimate of embryonic death is shown in the following sample calculations, and in Table 1 which gives data for 7,065 inseminations with unfrozen semen used one day after processing (Salisbury and Hart, 1970).

No. inseminations	7,065
Fertility at 30 days	80.3%
Fertility at 180 days	66.2%
Difference	14.1%

Assume ratio of normal 3-week cycles:normal multiple cycles due to missed heats = 3:1 (see text)

post-30-day returns due to missed heats

$$= \frac{(100 - 80.3)}{3} = 6.8\%$$

30-day to 180-day embryonic deaths

$$= (80.3 - 6.8) - 66.2 = 7.3\%$$

% embryonic death rate of 30-day conceptions

$$= \frac{7.3}{80.3 - 6.8} \times 100 = 9.9\%$$

These calculations show that the estimate of embryonic death calculated either as a percentage of the number of cows inseminated, or as a percentage of probable 30-day conceptions varies with the incidence of missed heats (Table 1). Pelissier

TABLE 1: THE EFFECT OF OESTRUS DETECTION EFFICIENCY ON ESTIMATES OF EMBRYONIC DEATH RATE

% Multiple Cycles (a)	*Fertility Decline (b) %*	*Difference due to Multiple Cycles (c)*	*Embryonic Death Rate (c)*	
			% All Cows	*% Eggs Fertilized*
16.7	14.1	9.9	4.2	6.0
20.0	14.1	7.9	6.2	8.6
25.0	14.1	6.8	7.3	9.9
28.6	14.1	4.9	9.2	12.2
33.3	14.1	3.9	10.2	13.4

(a) % of all return intervals due to missed heats (refer test example)
(b) 30-day Fertility — 180-day Fertility = 80.3% — 66.2% (Salisbury and Hart, 1970).
(c) Refer test example.

(1972) estimated that 1 out of 6 cows which failed to conceive had 6-week cycles because of missed heat periods. Data on first inseminations in the Illinois study (G. W. Salisbury, pers. comm.) show the incidence of double cycles in their cattle to be much higher than in New Zealand (Table 2). In fact, the percentage of returns within 180 days of insemination due to missed heats could lie between 25% (1 in 5 with double cycles) and 33% (1 in 4 with double cycles).

EMBRYONIC DEATH

The significance of embryonic death due to age of semen, sire or month of processing (Salisbury and Flerchinger, 1967;

TABLE 2: 1-49 DAY RETURN PATTERNS TO FIRST INSEMINATION IN ILLINOIS AND NEW ZEALAND DAIRY CATTLE

Return Intervals (days)	*% 1-49 day Returns*	
	Illinois	*N.Z.*
1-7	0.7	5.3
8-10	0.8	5.9
11-13	0.7	2.9
14-17	3.5	4.6
Short 1-17	5.7	18.7
18-19	16.4	12.8
20-21	25.0	25.6
22-24	19.3	21.9
Normal 18-24	60.7	60.3
25-33	7.7	8.9
34-49	26.0	12.1
Long 25-49	33.7	21.0

Salisbury, 1968; Salisbury and Hart, 1970) cannot be accurately assessed until a correction is made for missed heat periods. In addition, there are uterine factors unrelated to the sire. Biggers (1969) has shown the necessity to estimate these female factors in assessing the importance of embryonic death. Semen processing techniques may also be involved. The significant differences between sires in embryonic death rate reported by Salisbury *et al.* (1952) were based on inseminations with semen to which antibiotics had not been added. Willett and Ohms (1955) showed that specific antibiotics improved the below average non-return rates and reduced the delayed returns to service observed with some sires but did not alter the results for sires with above average fertility. The influence of pathogenic organisms may not be entirely controlled merely by the addition of antibiotics, as several studies with chilled semen have shown that semen used on the day of collection and processing had a lower 30-day non-return rate and a disproportionately greater decline to the 180-day non-return rate than the same semen used 1 day later (Salisbury, 1968). In our laboratory we have recently found that bacterial contaminants can be found in diluted semen at least for 8 hours after processing, but contamination levels are extremely low 24 hours later.

EFFECT OF FREEZING

The interactions between the storage time of frozen semen, the non-return rate and embryonic death are varied. Salisbury (1967) reported that when sperm were stored at $-79°$ C to $-88°$ C, fertility levels decreased with storage time, and that this decrease was associated with an increasing embryonic death rate. The effects were greatest for sperm collected during the summer months. Hafs *et al.* (1971) actually recorded an increase in fertility if the diluted semen, stored at $-195°$ C was used from 3 to 6 months following processing. The improved fertility obtained through using α-amylase or β-glucuronidase primarily occurred during this period.

The freezing process *per se* does not appear to influence the incidence of recorded abortions as Wijeratne and Stewart (1971) found that the abortion rates for fresh and frozen semen were 2.10% and 2.06%, respectively. This extensive study showed that there were significant breed differences in abortion rates but differences between sires within breeds were not significant when calculated as a proportion of conceptions.

HERITABILITY OF FERTILITY

There are consistent differences between sires in non-return rates. Shannon and Searle (1962) estimated the repeatability of fertility (49-day non-return rate to first insemination) between years was 0.69 ± 0.05. This same study also showed that the heritability of conception rate based on a sire-son regression was 0.55 ± 0.26. These authors suggested that it may be desirable to discriminate against sons of sires with low conception rate. The heritability estimates of most other reproductive or fertility parameters, particularly those relative to the cow, have been small (Hahn, 1969; Foote, 1970) probably because of the necessity to use large numbers of animals to account for binomial variation.

When there is sterility or infertility, numerous studies have implicated genetic factors related to specific anatomical or physiological defects (Beatty, 1970; Foote, 1970; Bishop, 1972). Bulls with such defects are usually quickly detected and removed.

RETURN-INTERVAL ANALYSES

There are only limited data on factors producing the consistent fertility differences observed in sires considered acceptable for use. Data from a recent analysis involving 41 bulls of 4 breeds used in the Auckland Herd Improvement Association in 1970 are summarized in Table 3. Only first in-

TABLE 3: MEAN 49-DAY % NON-RETURN RATE (N.R.) AND RETURN INTERVALS (± S.D.) FOR 41 BULLS AND CORRELATION COEFFICIENTS BETWEEN EACH INTERVAL AND N.R.

Non-return Rate (N.R.) or Return Interval (days)	*% Total Insemination*	*± S.D.*	*Correlation with N.R.*
1-7	1.7	0.5	—0.32
8-10	2.5	0.4	—0.43
11-13	1.0	0.2	—0.34
14-17	1.7	0.4	—0.42
Short returns (1-17)	6.9	1.0	—0.53
18-19	5.5	1.1	—0.91
20-21	10.1	2.5	—0.97
22-24	8.1	1.9	—0.94
Normal returns (18-24)	23.7	5.3	—0.98
25-33	3.2	0.3	—0.06
34-49	4.3	0.5	—0.25
Long returns (25-49)	7.5	0.5	—0.26
N.R.	61.9	5.9	—

seminations with semen diluted in caprogen (Shannon, 1965) containing catalase and used on the day following processing were considered. The average number of inseminations per sire was 9308. The mean non-return rate was 61.9% (SD = ± 5.9%). Return-interval analyses showed that fertility differences between bulls were largely accounted for by the incidence of normal return intervals of 18 to 24 days ($r = -0.98$; $P < 0.001$). Although the correlation between non-return rate and long-return intervals (25 to 49 days) was not significant ($r = -0.26$), these data do not disprove the importance of embryonic death as a factor influencing some sires' results. The importance of embryonic death will be primarily related to the proportion of fertilized eggs rather than number of cows inseminated.

The factors most likely to influence the incidence of normal returns to service could include failure of fertilization, fertilization followed by early abnormal development or abnormal egg transport, fertilization with normal development and egg transport but failure to implant in the uterus, or rapid death of the embryo following the initiation of implantation. Technical factors such as site of semen deposition may influence fertilization rate (Moller *et al.*, 1972) and also egg transport, as Holst *et al.* (1972) found the handling of the ovine reproduc-

tive tract accelerated ovum transport. The primary effect of the sire should be reflected in the fertilization rate.

FERTILIZATION RATE

Kidder *et al.* (1954) compared results in heifers inseminated with unfrozen semen from sires with high, average or low non-return rates and found the respective fertilization rates to be 100% (22 out of 22), 82.1% (23 out of 28) and 71.4% (10 out of 14). The heifers were slaughtered 3 to 5 days from the commencement of heat. Bearden *et al.* (1956) slaughtered heifers at 3 days post-oestrus and found that the fertilization rate when using semen from "high" fertility sires was 96.6% (28 out of 29). The comparable result for "low" fertility sires was 76.9% (20 out of 26). Both groups concluded that the primary differences between sires of differing non-return rates was failure of fertilization.

The interval between insemination and ovulation is another factor influencing fertilization rates. Barrett and Casida (1946) found maximum conception rates with inseminations from 3 to 20 hours after a cow was first noticed in heat. In more extensive studies, Trimberger and Davis (1943) and Trimberger (1948) showed that conception rates were highest for inseminations made either during the latter half of an 18-hour oestrous period or during the 6 hours immediately following the cessation of oestrus. The poorer results for early oestrous inseminations were the consequence of sperm death prior to ovulation. Even though some sperm may be rapidly transported to the site of fertilization following insemination (Van-Demark and Moeller, 1951), inadequate sperm numbers as well as insufficient time for capacitation could contribute to the reduced fertility observed for inseminations within 6 hours of ovulation. Kirton *et al.* (1968) and Hafs *et al.* (1971) have suggested that improved non-return rates obtained through the use of enzyme additives such as amylase and β-glucuronidase could be the consequence of reducing capacitation time, although Mahajan and Menge (1966) could not demonstrate a delay in fertilization ability which could be attributed to this phenomenon.

BETWEEN-EJACULATE VARIATION

Since a sire's non-return rate between seasons is highly repeatable and this same reproductive parameter is inherited (Shannon and Searle, 1962), a simple genetic relationship could involve consistently low non-return rates from all batches of semen processed from a sire with below-average fertility. However, Macmillan (1970) noted significant non-

return rate differences for different ejaculates from the same sire. These differences were apparently a characteristic of the ejaculate as subsequent experiments showed that mixing semen from a sire's first and second ejaculates reduced residual variation on a within-bull basis (Macmillan and Watson, 1971). An analysis of data for separate ejaculates from 34 bulls showed that sires of below-average fertility exhibit greater non-return rate variation between ejaculates than sires of average or above-average fertility.

Within a population there will be a maximum obtainable non-return rate set by factors influencing female fertility, technician ability, accuracy of oestrus detection and the interval from detection to insemination and to subsequent ovulation. Therefore a sire cannot maintain a high non-return rate unless results for most of his batches are close to the population limit. In contrast, a sire with a low non-return rate can either be consistently low because of genetic factors influencing the fertilization capacity of a proportion of the sperm which he produces, or some batches can be good and others poor because of varied susceptibility of sperm from different batches to imperfections in processing or in the extender. Our data show that increased between-batch variation is one factor associated with sires with below-average fertility.

TIME OF INSEMINATION

During 1971, 60 Taranaki farmers classified all their artificially-inseminated cows into one of four categories relative to time of first observation of oestrus and the subsequent duration of oestrus. These categories were:

(1) Cows first seen in oestrus at the a.m. milking which were inseminated following that milking but were still in oestrus at the next p.m. milking. Most of these cows would be inseminated in early oestrus.

(2) Cows first seen in oestrus at the a.m. milking which were inseminated following that milking but were not seen in oestrus at the next p.m. milking. Most of these cows would be inseminated around mid-oestrus.

(3) Cows first seen in oestrus at the p.m. milking which were still in oestrus at the next a.m. milking following which they were inseminated. Most of these cows would be inseminated during late oestrus.

(4) Cows first seen in oestrus at the p.m. milking which were not in oestrus at the next a.m. milking following which they were inseminated. Most of these cows would be inseminated in post-oestrus.

Almost all farmers had a 12.5 to 13.5-hour interval between the p.m. and a.m. milking and most inseminations with semen diluted in caprogen (Shannon, 1965) containing catalase (4.5 μg/ml) were made between 9.00 a.m. and 11.30 a.m. The results support the conclusions of Trimberger and Davis (1943) as highest non-return rates were obtained for late oestrous inseminations (Table 4). Accurate comparisons can be made only after correcting for differences in the incidence of short return intervals (1 to 17 days) which partly arose through errors in heat detection. Cows classified in the early and late oestrous categories were seen in oestrus at two consecutive milkings and therefore fewer errors were made. The simplest alternative is to compare 18- to 49-day non-return rates.

Inseminations within each category were re-classified into one of three sire-fertility groups. Each extensively-used sire was classified as being of above-average, average or below-average fertility, based on results obtained throughout Taranaki during the experimental period. The results clearly show that the time of insemination was least important for the group of sires with above-average fertility (Table 5). Sires with average fertility had lower results for early oestrous inseminations (74.3% v. 62.8%). Below-average sires had lower results than the above-average sires for all but the post-oestrous inseminations, and as the average time interval from insemination to ovulation increased the results for below-average bulls declined. The data lead to the conclusion that sperm from sires with below-average fertility have a shorter *in vivo* life span and die before ovulation. The relatively large differences in non-return rate due to the effect of time of insemination suggest that *in vivo* sperm livability is a major factor influencing a sire's fertility.

IN VIVO SPERM LIVABILITY

The recording techniques used were not complicated and even if the primary distinction is only to identify cows first seen at the p.m. milking, significant differences between sire groups can be developed to specifically test the nature of a response to an extender additive. Kirton *et al.* (1968) and Hafs *et al.* (1971) added enzymes which may have increased rate of capacitation. This would have been proved if all groups of bulls had improved results with post-oestrous inseminations. It is possible that, if this had occurred, differences with early oestrous inseminations would be increased as sperm appear to lose their fertilizing capacity more rapidly following capacitation when sperm metabolic rates increase (Bedford, 1970). Capacitation may occur rapidly in the bovine uterus (Mahajan

TABLE 4: THE EFFECT OF STAGE OF OESTRUS AT INSEMINATION ON 1- TO 49-DAY AND 18- TO 49-DAY NON-RETURN RATES

Stage of Oestrus at Insemination	*No. of Observations (a)*	*No. Cows*	*Return Intervals (days)* *1-17 (%)*	*18-24 (%)*	*25-49 (%)*	*% Non-return Rate* *1-49 day*	*18-49 day*
Early	2	821	3.2	27.8	4.3	64.8	66.9
Mid	1	3383	6.3	21.5	6.8	65.4	69.8
Late	2	822	3.2	19.2	5.4	72.3	74.7
Post	1	2590	5.6	20.2	6.3	68.0	71.9
Total	—	7616	5.4	21.5	6.2	67.0	70.8

(a) Cows seen at 1 or 2 consecutive milkings (refer to text).

TABLE 5: THE EFECTS OF STAGE OF OESTRUS AT INSEMINATION AND SIRE FERTILITY LEVEL ON 18- TO 49-DAY NON-RETURN RATES

Stage of Oestrus at Insemination	*Sire Fertility Group*		
	Below Average	*Average*	*Above Average*
Early	59.0(a)	62.8	74.3
Mid	65.7	70.7	71.8
Late	71.8	75.4	78.3
Post	73.1	71.5	73.4
Total	68.3	70.6	73.3

(a) 18- to 49-day % non-return rate.

and Menge, 1966) and there may be some advantage in reducing rather than accelerating capacitation rate (Macmillan, 1970).

O'Reilly *et al.* (1972) used heterospermic inseminations to evaluate the effects of processing techniques on the fertility of rabbit semen. The number of offspring to each breed of sire was probably a reflection of differences in *in vivo* sperm livability. Miller *et al.* (1969) and Dziuk (1970) used double mating techniques in rabbits, gilts and ewes. The recording of the time intervals from oestrous observation to insemination could be a practical alternative in studying factors which influence *in vivo* sperm livability in cattle.

The time intervals in the Taranaki study involved 6- to 8-hour periods and therefore relatively small differences *in vivo* livability can have major effects on fertilization rate. This may explain the poor correlations between *in vitro* sperm livability and non-return rate. An increase in sperm livability at 37° C of from 68.3 to 104.8 hours following the addition of catalase to caprogen did not significantly increase non-return rate (Macmillan, 1970), although in more extensive studies there was a small but significant increase (New Zealand Dairy Board, 1968). Semen diluents must maintain sperm prior to insemination as well as preserving the metabolic integrity of the sperm once they move out of the diluent following insemination. Important factors which influence this metabolic integrity may include the relative toxicity of the bull's seminal plasma (Shannon, 1965), the rate of release of amino-acid oxidase following capacitation or death (Shannon and Curson, 1972), or the effect of a seminal nucleosidase on sperm levels of NAD prior to insemination (Bistocchi *et al.*, 1968). Brooks and Mann (1971) showed the importance of NAD for the metabolism of motile ram and boar sperm, yet bull seminal plasma contains a nucleosidase which can destroy 60 to 70% of the NAD levels of epididymal sperm within minutes of ejaculation.

We have recently found marked differences in the concentration of this enzyme between sires. Results to date suggest that bulls with above-average non-return rates have little or no seminal nucleosidase.

FUTURE RESEARCH

If all sires of average or below-average fertility could be raised to the levels attained by sires of above-average fertility, non-return rates could be increased by at least 3%. Improved *in vivo* sperm livability will definitely facilitate the achievement of this objective. But the design of experiments to test for such improvement must take account of probable bull by treatment interactions as well as the fact that there can be significant differences in the non-return rates between ejaculates from the same sire. Split-ejaculate techniques should always be used and preferably an attempt made to assess the interval from oestrus detection to insemination. Factors which influence farmers' accuracy in oestrus diagnosis must also be considered in assessing differences in fertilization rate or embryonic death.

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