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Sperm numbers, semen age and fertility in fresh and frozen bovine semen

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ABSTRACT

The consequence of a seasonal mating pattern is that a significant amount of semen from the top sires is required during this intensive mating period to satisfy the demand. To utilise semen from these top sires efficiently, liquid semen technology has been extensively used in New Zealand. This paper describes the essential physiological differences between liquid and frozen semen and their relative advantages and disadvantages. Alternative technologies such as freezing semen in bulk and later rediluted for use as liquid semen can be used to overcome the constraints of an intensive mating season.

Keywords: Liquid semen; sperm numbers; frozen bovine semen

INTRODUCTION

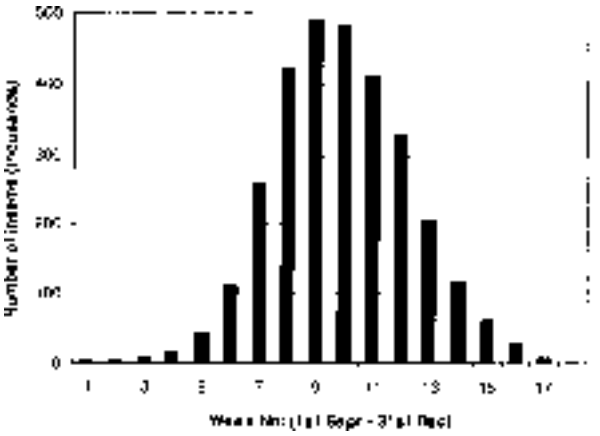
Genetic gain in the New Zealand dairy herd is dependent upon two processes: usage of the top genetic merit bulls and the selective rearing of high breeding index calves as replacements. To this end, artificial insemination has remained the main vehicle for the rapid dispersal of valuable genes within the dairy industry. The herd average breeding index (BI) is a good indicator of the genetic progress made in that herd relative to a base BI value established in 1960. A 37% increase in genetic merit has been recorded and nationally, it is estimated that 30% of this genetic gain is exclusively attributable to the use of artificial insemination (P. Shannon, unpublished information). Much of the advancement of artificial insemination is singularly due to advances in semen technology which has allowed the dairy industry to maintain a steady level of genetic progress. The requirements of large volumes of semen from high genetic merit bulls during an intensive mating period has meant that along with the standard frozen semen procedure, an indigenously developed liquid semen system has worked well to spread the top bulls around the national dairy herd (Shannon, 1978, Curson *et al* 1991). The immediate advantage of this system is that fertility is maintained with low numbers of total sperm in the inseminate and thereby increases the coverage of the bull.

THE BREEDING SEASON

The distribution of inseminations from the 1st of September to the 31st of December is shown in Figure 1. In this period close to 3 million inseminations are conducted. The peak usage of semen is during the weeks between October 27th and November 10th where close to 60% of the dairy cattle population in New Zealand are inseminated. During these days the average despatch of liquid semen range from 45,000 to 75,000 individual doses for a three day period of use. To satisy this semen demand, three technologies are extensively used;

- Fresh semen (Long Last Liquid® semen) stored at ambient temperature (15°C to 21°C) and used over a three day period (Curson *et al*, 1991). This accounts for 80% of the inseminations.
- Frozen semen - 15% of the inseminations.
- Bulk frozen semen rediluted as fresh - 5%.

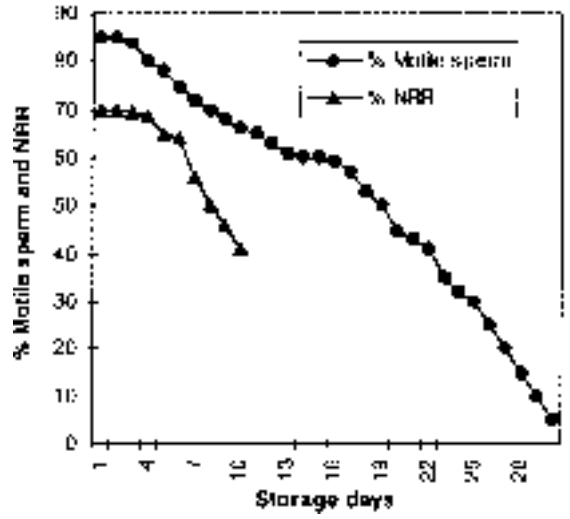
FIGURE-1: The distribution of inseminations in New Zealand during the spring mating period (1st September - 31st December).



PHYSIOLOGICAL DIFFERENCES FRESH AND FROZEN SEMEN

The effect of storage of semen on sperm motility over a thirty day period at ambient temperature is shown in Figure 2. This figure also shows the change in percentage non return rate (%NRR) over a ten day period at ambient temperature. The drop in the percent of motile sperm is not significant even at 14 days after storage at ambient temperature, about 60% of the sperm are still vigourously motile. *In vivo* fertility as measured by NRR shows a different pattern. Fertility is maintained for the first three to four days after dilution (69.9 ± 1.2%) following which there is a steady drop in NRR till day-10 (41.5 ± 3.7%, $p<0.05$).

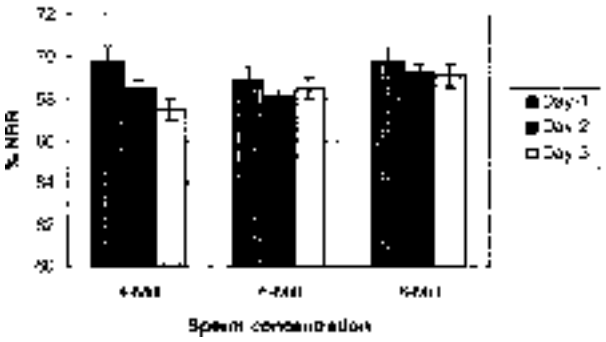
FIGURE 2: The effect of ambient temperature (18(C - 21(C) storage in CAPROGEN® on sperm motility and % non return rate.



The other important factor that does have a significant influence on the NRR is the dose rate or absolute sperm numbers in a given inseminate. This relationship between semen quantity (sperm numbers) and semen quality (fertilising potential) was first described by Salisbury and VanDemark (1961). The main point in this concept is that up to a threshold level, fertility is under the inflence of sperm numbers. This threshold varies between bulls and once this level is attained, fertility is unaffected by further increases in sperm concentration. This has been adequately demonstrated in field trials where some of the inherent fertility differences of different bulls can be compensated for by altering the absolute numbers of sperm in the inseminate (den Daas, 1992., Shannon and Vishwanath, 1995). The effect of sperm numbers being able to influence NRR is a defined compensable element and increasing sperm concentration does alter the probability of fertilisation (Shannon and Vishwanath, 1995). The change in NRR at different dose rates in fresh semen is shown in Figure 3. The probability of fertilisation is quite high on the first day of use at all three sperm concentrations (4, 6 and 8 million/ml) and they are not significantly different from each other. On the contrary, with storage, the NRR is lower on days 2 and 3 at the lowest sperm concentration of 4 million / ml ($p<0.05$). The reasons could be:

- Probability of fertilisation decreased due to lower sperm numbers.
- Effect of dilution compounding the sperm ageing effect.
- Bull x dose rate interaction.

FIGURE 3: Change in % non return rate at different sperm concentrations in liquid semen inseminated on days 1 to 3 after collection.

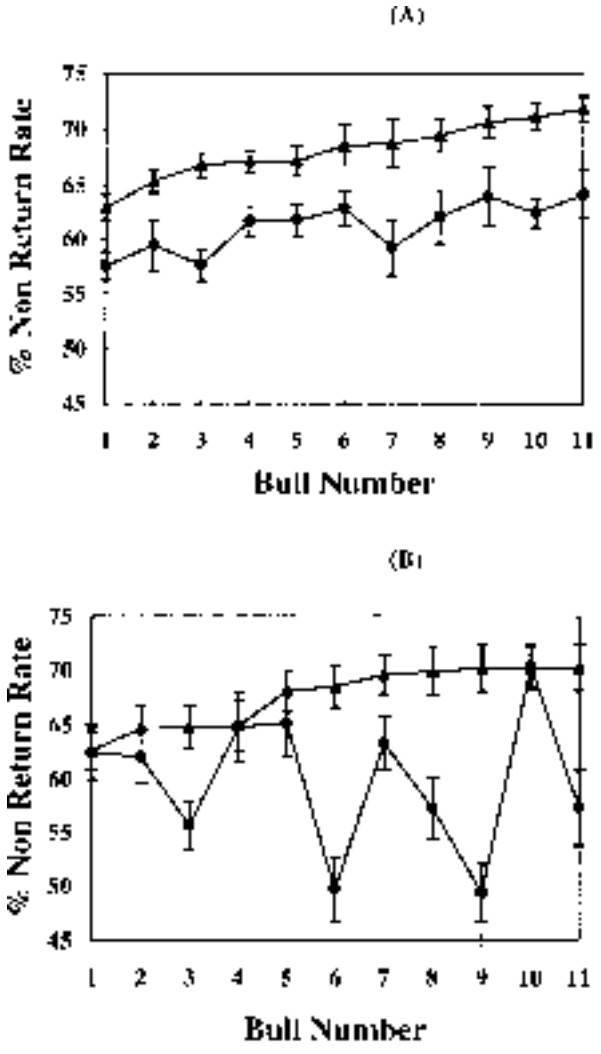


Differences Liquid vs frozen semen, effect of sperm numbers

Bulls differ in their inherent fertility and follow similar fertility trends whether they are diluted and stored as liquid or frozen semen. This effect is shown in figure-4 and the %NRR of fresh and frozen semen for the same 11 bulls at optimum sperm concentration (liquid 2.5 million/ inseminate, frozen 20 million/inseminate) range between 62% and 72% (Shannon and Vishwanath, 1995). When sperm concentrations are dropped to sub optimum levels, (liquid 0.5 million/inseminate, frozen 5 million/inseminate), fertility drops by an average of 7% in liquid semen and by 7.9% in frozen . There was a significant bull x dose rate interaction in the frozen semen system which was not seen in the liquid system ($p<0.01$). This clearly highlights the fact that semen from different bulls inherently differ in

their suitability for cryopreservation as measured by their fertility success. Further evidence suggests that this could be due to a reduced probability of fertilisation or an altered pattern of frozen semen survival in the female reproductive tract (Shannon and Vishwanath, 1995).

FIGURE 4: Variation in NRR between bulls at optimum (▲) and suboptimum (●) sperm concentrations in the fresh (A) and frozen (B) treatments. (Reproduced with permission from Shannon and Vishwanath, 1995).



ADVANTAGES - DISADVANTAGES, FRESH VS FROZEN SEMEN

The relative advantages and disdvantages in the field usage of fresh and frozen semen is listed in Table 1. The most obvious advantage is one of sperm numbers and this has been the main impetus in further refining this technology for extensive use in New Zealand. An average ejaculate of 5 ml containing 1.5 billion sperm / ml will yield between 350 and 500 frozen semen straws at a dose rate of 15 to 20 million sperm / inseminate. On the other hand, the same ejaculate as liquid semen will yield 7,500 straws at a dose rate of 1 million / inseminate and 3,750 straws at the higher sperm concentration. The storage costs of fresh semen is very low and sire utilisation is greater than 90%. The obvious disadvantage with fresh semen is the limited shelf life. The most important advantage of frozen semen is the long term storage capability. The biggest disadvantage is the the high sperm numbers required to maintain the same level of fertility as liquid semen.

TABLE 1: Relative advantages and disadvantages of field usage of liquid and frozen semen.

Fresh	Frozen
Advantages	Advantages
• Low sperm numbers	• Long term storage
• High sire utilisation	• Flexibility of use
• Inexpensive storage.	Disadvantages
• Ease of use in the field.	• High sperm numbers.
Disavantages	• Expensive to store
• Limited shelf life	

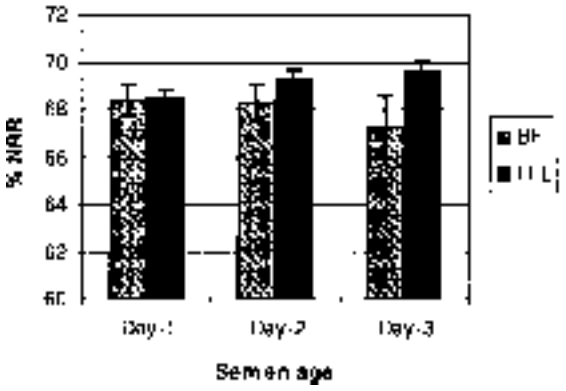
BULK FROZEN SEMEN

The concept of rediluted deep frozen technology was first explored by James and Fyvie (1955). The idea was to develop it as a convenient method to harness the out of season production potential of a bull. The initial trials were not very successful and the fertilising ability of rediluted semen could be retained for only a few hours. This technique was later successfully modified for field usage (Shannon, 1972, Macmillan *et al* 1978). In this system semen was frozen in individual straws at high sperm concentrations and subsequently rediluted upon thawing into CAPROGEN® (Shannon, 1965) and used as liquid semen. This technology was efficiently used for a number of years in some isolated areas where the technician diluted the contents of one straw and held the contents at ambient temperature. Inseminations were done using a syringe pipette and an inseminate volume of 0.5ml. This process has been improved so that large volumes of concentrated semen (5 - 25 ml) can be bulk frozen (Patent applied) and rediluted in the production laboratory on the days required for use. The semen is then despatched as standard liquid semen and used on days 1 and 2 after redilution. The sperm concentration of the rediluted material is between liquid and frozen systems and range between 6 - 10 million sperm/inseminate. The results with Bulk Frozen Semen is shown in Figure 5. The %NRR for the first two days of use was not significantly different between the two semen types but was significantly lower on day-3 of use with bulk frozen semen ($p<0.05$). The two aspects that could potentially affect this system is the profound bull x sperm concentration interaction which will determine the final sperm concentration in the inseminate and the bull x semen age interaction which will limit the number of days semen can be used in a diluted state in the field.

SUMMARY

The use of liquid semen allows high utilisation of individual sires. This is possible because of the low sperm numbers required to maintain fertility and the extended

FIGURE 5: Comparison of non return rate between bulk frozen semen and liquid semen on days 1 to 3 after thawing / rediluting and collection respectively.



shelf life of up to four days of use in the field. If the semen age effects can be combated, the efficiency and utilisation of liquid semen could be increased quite significantly. The essential difference between liquid and frozen semen lies in the response of bulls to sub-optimum dose rates. Whilst all bulls respond similarly to sub-optimum dose rates with fresh semen, the response varies greatly when semen was frozen. This means that higher than required dose rates are recommended for frozen semen because of the possibility of a bull x dose rate interaction. The use of bulk frozen semen rediluted to cover those days of high semen demand is another option to combine the convenience of frozen semen technology with the efficiency of liquid semen.

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Reproductive efficiency in lactating dairy cows

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ABSTRACT

Both genetic improvement and milk yield of dairy cows are dependent on a high reproductive performance. Under normal situations, oocyte fertilisation rates following insemination at the appropriate time are high (>90%) and gross genetic defects in embryos are estimated to be around 10%. This means that the biological limit of conception rate should be around 80%. Such a performance has rarely been realised in practice, with 60% being a normal target for lactating cows in New Zealand dairy herds. At a herd level, reproductive performance is best measured by the percentage of cows pregnant within a period after start of the breeding season and this is determined by the combination of submission rate and conception rate. Major factors that affect reproductive performance in New Zealand dairy herds include anoestrus, errors in heat detection, fertilisation failure and embryo mortality. Given adequate nutrition, herd management is undoubtedly the major determinant of reproductive efficiency. The low reproductive efficiency in dairy cows creates a bottleneck to genetic improvement and is a major factor affecting milk production efficiency.

Keywords: fertility; reproductive efficiency; dairy cattle.

INTRODUCTION

Under the New Zealand seasonal dairy production system, the reproductive performance of dairy cows is undoubtedly one of the most important determinants of production efficiency and profitability. Both milk production and genetic improvement of dairy cows are heavily dependent on a high reproductive efficiency. Reproductive performance affects