

## Has the Fertilizing Capacity of Bovine Spermatozoa Changed?

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### Introduction

An evaluation of historical data has been a challenge because there is always the possibility that something in the data set, that is not obvious, will be misinterpreted. This is especially true when attempting to evaluate fertility. Also a large amount of data is needed before one should make any conclusion. The topic of today's discussion is timely because there are many frustrated people in the dairy industry looking for answers for their reproductive problems. Some attempt to fix blame rather than try to solve the problem. From the little that I know about Dr. Petersen, I am sure that he would have loved to have been involved in a symposium like we are having today. Before addressing the topic assigned, I would like to review three basic principles about fertility that have been taught by three of the early pioneers in AI. These principles might be of some use later in the discussion session of this symposium.

First, in evaluating fertility, several variables must be considered. One of the early pioneers in AI at ABS, Dr. David E. Bartlett a graduate of U of Minnesota, developed an Equation of Reproduction [1] that he used when training technicians. This equation has stood the test of time when it comes to understanding reproductive efficiency. The equation states that there are four independent factors that affect reproduction. These factors are:

- A = Herd members detected in heat and inseminated (%)
- B = Inseminator efficiency (%)
- C = Fertility level of the herd (%)
- D = Semen fertility level (%)

Percentage of pregnancies resulting from AI is the product of the four factors and not the average of the four factors. When technicians left his class, each had a good understanding of this equation and what it meant. The equation is still valid today. I will concentrate only on the semen factor.

Another pioneer in reproduction and AI was Dr. E.F. Graham, another graduate and Distinguished Professor of the U of Minnesota. He was involved in a lot of great research and one of his classic papers given to the industry was entitled "The Usefulness of Useless Data-Field Tests and Responsibilities." [2] Upon reading this paper, one will feel the frustrations that he was having in trying to get good fertility data. He even convinced one of the AI organizations, that he was working with, to send out over 1,200 insemination units that only contained either extender alone or dead sperm in extender to prove his point about the inaccuracies of non-return data. To make sound judgments we need good data.

A third pioneer in bovine reproduction, Dr. Robert Foote Professor Emeritus at Cornell University, also had concerns about fertility information. He brought to our attention the reason we need a large number of inseminations in order to get accurate fertility information. This is necessary due to binomial variance resulting from the fact that a cow is either pregnant or open. [3] The effect of sample size on the 95% confidence interval is shown in Table 1. It is probably fair to say that many of the conclusions drawn from small data sets have been flawed because of binomial variation.

As I attempt to discuss the topic that I have been assigned for this symposium, there are two areas

regarding bull fertility that need to be discussed. They are 1) AI industry changes that have enhanced fertility of AI bulls and 2) Physiological changes in bull spermatozoa that might indicate a change in fertility.

### **AI Industry Changes That Enhanced Bull Fertility**

Since the first AI center in the USA started in 1938, [4] there have been several changes that have either directly resulted in increased fertility of semen or indirectly through the decisions that have been made. Summaries of the major changes that have enhanced fertility are found in Table 2. These changes fell into three categories:

1. Controlling reproductive diseases
2. Technical breakthroughs
3. Early development of an industry organization that shared ideas and linked with academia.

In addition to the changes shown in Table 2, during this same period, there have been volumes of research dedicated to understanding both the physiological and morphological characteristics of spermatozoa that identify them as being able to fertilize oocytes.

These changes have had a tremendous positive impact on the fertilizing capacity of the semen that is used today. The elimination of transmission of reproductive diseases through AI has also had far reaching implications on the cattle industry. It is impossible to assess what the full impact of these improvements would be, however; by adding the increases found from individual research trials one can get some idea that the effect would be great. From a technological standpoint, the dairy industry is receiving the highest quality semen ever produced.

### **Has There Been Physiological Spermatozoal Changes In The Bull Population Since Initiation Of AI That Could Affect Fertility?**

To attempt to answer this question I am going to use some ABS historical information as there is very little published data in this area. The measurement of scrotal circumference of bulls has been a routine practice at AI organizations for over 20 years. Published data [22] collected on young Holstein bulls from 1982-1986 and two unpublished data sets from ABS, on young Holstein bulls, one collected from 1988-1993 and the other from 1999-2002, are shown in Figure 1. This data indicates that growth patterns of the testicles over the 20 years are very similar.

ABS has used an objective photographic technique [23] for measuring sperm motility since 1954. Over a time period of this length, I am sure that there would have been some slight modifications in this technique but, as far as I know these modifications have been minor. Figure 2 shows the percent progressively motile spermatozoa (post-thawed) from all mature Holstein bulls that were collected during a particular year. The average percent motility has been calculated from 1958-2002. Note that the average percent motility started to increase in 1981. During this time frame two things changed. The first was the conversion to the straw, which took place over several years. The second was thawing all semen, whether packaged in ampules or straws, in 37 C water. Thawing semen at 37 C increases the percentage of spermatozoa that survive the freezing and thawing process. In order to establish that it was not the photographic techniques that had changed, we re-photographed 144 samples in 1999 that had been originally photographed in 1980. Table 3 indicated that no motility differences were found over the two time periods ( $P>0.01$ ). Therefore the differences that were observed in Figure 2 were due to methods of freezing and thawing spermatozoa and not a change in the bulls being brought into the stud.

Evaluation of sperm morphology predates AI in cattle. [24] Several classifications of abnormal sperm morphology were in use by the time AI was initiated. ABS started to use Blom's classification shortly after his proposal in 1972. [25] This classification has been used at ABS since that time with only minor modifications. Figure 3 gives the percentage of spermatozoa with primary morphological abnormalities as well as the percent total morphologically abnormal spermatozoa from all mature Holstein bulls that were collected during a particular year. Distinct changes in the percentage of primary and total abnormal spermatozoa were observed starting in 1990. The explanation for this hinges on the fact that new microscopes, with better optics, were purchased resulting in technicians being able to observe smaller differences in spermatozoa. After a retraining session, it was decided to record what was now being observed and change the parameters for rejection of collected semen based on abnormalities of spermatozoa. One other change in technique was made in 1997. Evaluation of sperm morphology was made using wet mount preparations rather than staining.

To verify that the observed increase in abnormal spermatozoa was due to better optics and retraining, 25 samples of semen frozen prior to and after 1990 were re-evaluated in 2002. Three technicians evaluated each sample that had been coded to eliminate any possible bias. As shown in Table 4, there was a significant ( $P < 0.01$ ) increase in the percentage of spermatozoa that were classified abnormal for samples where the original evaluation was made prior to 1990 but no difference ( $P > 0.01$ ) for those that had been evaluated after 1990. Therefore, it would appear that no change in the morphological structure of spermatozoa over the period that was evaluated has occurred.

### **Has The Number Of Spermatozoa Necessary To Obtain Optimum Fertilization Changed?**

Salisbury and VanDemark put forth a theory that it was the number of viable spermatozoa that affected fertility. [26] They proposed that fertility would increase as the number of viable spermatozoa increased until a threshold for optimum fertility is reached. Sullivan and Elliott [13] validated this theory by inseminating three different numbers of viable spermatozoa that had been frozen in ampules and thawed in iced water. Pace et.al [16] further defined this relationship using an exponential model which predicts a limiting fertility rate (asymptotic value) as the number of viable sperm increases. They also found that merely increasing the number of spermatozoa inseminated within the range of this study did not bring the technicians with a lower nonreturn rate up to the level of those with a higher rate. A later study by den Daas [27] confirmed these findings. In both of these studies a number of different concentrations of viable spermatozoa were inseminated after the semen had been frozen in plastic straws and thawed in 35-37 C water.

Seidel et al., [28, 29] found that the number of spermatozoa needed for insemination is dependent upon semen placement. They found the same fertility inseminating 500,000 total spermatozoa at the tubouterine junction as inseminating 10,000,000 total spermatozoa in the uterine body. They also believe (unpublished observations) that more sperm are needed when applying this deep insemination technique to cows vs. heifers.

Shannon and Vishwanath [30] have shown that more re-extended frozen than unfrozen spermatozoa were needed to obtain the same fertility (Table 5). Their data clearly shows that it takes four to five times more re-extended frozen sperm to produce the same fertility as with non-frozen sperm. It is interesting to note that non-frozen spermatozoa do not give higher fertility than frozen spermatozoa if enough frozen spermatozoa are inseminated. This author could not find any comparable studies looking at conventional frozen semen and unfrozen semen in order to evaluate how many more frozen sperm are necessary to obtain the same fertility.

In summary, while no direct comparisons can be made between sperm numbers inseminated during the developing years of AI and current numbers. Recent studies have shown that the asymptotic value for the number of sperm inseminated is much lower than were being used in early AI years. It also

appears that any sperm number studies to find optimum numbers will need to account for insemination location and parity of those being inseminated.

### **Effect Of Inbreeding On Semen Quality**

To assess the effect that inbreeding is having on semen quality of young bulls selected for AI, mean % post-thaw motility, mean % spermatozoa with primary abnormalities, and mean % total morphologically abnormal spermatozoa were evaluated for 1,968 young Holstein bulls coming to ABS between 1994 and 2002 (ABS unpublished data). Inbreeding values for these bulls ranged from less than 1 to >19%. The R<sup>2</sup> values for the regression of % inbreeding on % post-thaw motility, % primary abnormalities, and % total abnormal spermatozoa were 0.0002, 0.0062, and 0.0017, respectively. So far inbreeding has had little effect on spermatozoal quality measurements. However, this does not mean that animals with high inbreeding values will not have fertility problems when mated to each other.

### **Concerns For The Future**

Artificial insemination is in its 65<sup>th</sup> year of existence in the USA. Great strides have been made in semen preservation. Some would say that it has become a mature industry; yet, there are still concerns and opportunities. Here are few that I see:

- Who is going to train the next generation of technical staff on a dairy operation to use proper techniques and understand causes for the dairy's reproductive efficiency?
- With changes in the way semen is delivered to our customers, how are we going to assure quality of product upon arrival to the dairy?
- There will be challenges to quickly identify genetic recessives that affect reproduction.
- As the size of dairy operations increase, there is going to be an even greater need to understand the dynamics of animal health and environmental conditions.
- There still needs to be someone to sort out, "The usefulness of useless data." There are too many scientific papers being published that are useless because the number of observations is too small. This confuses dairymen who are not trained to sort out the information.

### **Conclusions**

There have been major advances in the preservation of bull spermatozoa in the last 65 years. These advances have resulted in a highly fertile product to be used in AI. While the genetic selection pressure has been high in determining which bulls will enter into AI programs, this pressure has not altered the quality of the spermatozoa being produced. As we continue in our efforts to solve the reasons for the downward spiral in fertility of our dairy cow population, a quote from Dr. W.E. Petersen, whom we are honoring at this symposium today, might be in order: "It should be recognized that there are some disadvantages connected with artificial insemination even if the work is done as is essential, by a skilled technician. 1) The owner must detect the cows that are in heat and report to the insemination service in time for insemination. Failure to do this is the most common cause of delayed breeding." [31]

From my prospective the quality of the spermatozoa produced by bulls has not changed during the time AI has been used. However, enhancements in cryopreservation of spermatozoa have allowed production of frozen spermatozoa with the highest fertilizing capacity that has ever been available to dairy operations. In order to realize the full potential of this technology, AI organizations have the responsibility to incorporate these fertility enhancements into their products.

Table 1: How numbers of inseminations affect fertility estimation.

Number of Inseminations	95% Confidence Interval* for % Conception
10	$\pm 29.0$
50	$\pm 13.0$
100	$\pm 9.2$
300	$\pm 5.3$
500	$\pm 4.1$
1000	$\pm 2.9$
5000	$\pm 1.3$
10000	$\pm 0.9$

\* The values assume that all of the error variance is due to binomial distribution

Table 2: AI sire fertility enhancement milestones (1938 – 2002)

Decade	Fertility Enhancement
1930s	-First AI center started [4] -Discovered beneficial effects of egg yolk extender [5]
1940s	-National Association of Animal Breeders (NAAB) established* -Penicillin and streptomycin added to semen [6, 7]
1950s	-First calf born from frozen semen [8] -Minimum health standards established for bulls in AI [9] -Liquid nitrogen tank produced [10]
1960s	-Added polymyxin sulfate to control Vibrio Fetus [11] -First NAAB technical conference* -Semen placement retraining started [12] -Viable sperm necessary for optimum fertility [13]
1970s	-Conversion from glass ampules to plastic straws for frozen semen [14] -Conversion from thawing in iced water to 37C water [14] -Changed antibiotic cocktail to control mycoplasma [15] -Certified Semen Services (CSS) organized by NAAB*
1980s	-Identified number of viable sperm necessary for optimum fertility for sperm packaged in plastic straws and thawed in 37C water [16] -Updated antibiotics used in frozen semen [17] -Identified bulls with a recessive gene for DUMPS [18]
1990s	-Started testing for persistently infected BVD bulls [19] -Identified bulls with recessive gene for BLADS [20]
2000s	Identified bulls with recessive gene for CVM [21]

\*National Association of Animal Breeders (NAAB)  
P.O. Box 1033, 401 Bernadette Dr., Columbia, Missouri 65203

Table 3: Re-evaluation of 144 samples for percentage progressively motile post-thawed spermatozoa that had been stored in liquid nitrogen tanks for 19 years.

	Year of Motility Evaluation	
	1980	1999
<b>% Progressive Motility</b>	<b>33.8<sup>a</sup></b>	<b>34.2<sup>a</sup></b>

<sup>a</sup> within a row, means with uncommon superscripts differ (P<0.01)

Table 4: Re-evaluation of Sperm Morphology

	Year of Cryopreservation			
	1973 – 1989		1990 – 2002	
	Classification of Abnormality		Classification of Abnormality	
	Primary (%)	Total (%)	Primary (%)	Total (%)
<b>Original Evaluation</b>	<b>2.9<sup>a</sup></b>	<b>5.3<sup>a</sup></b>	<b>10.6<sup>a</sup></b>	<b>14.0<sup>a</sup></b>
<b>Re-evaluation 2002</b>	<b>10.8<sup>b</sup></b>	<b>14.4<sup>b</sup></b>	<b>9.2<sup>a</sup></b>	<b>12.9<sup>a</sup></b>
<b>Difference</b>	<b>+7.9</b>	<b>+9.1</b>	<b>-1.4</b>	<b>-1.1</b>

<sup>a, b</sup> within columns, means with uncommon superscripts differ (P<0.01)

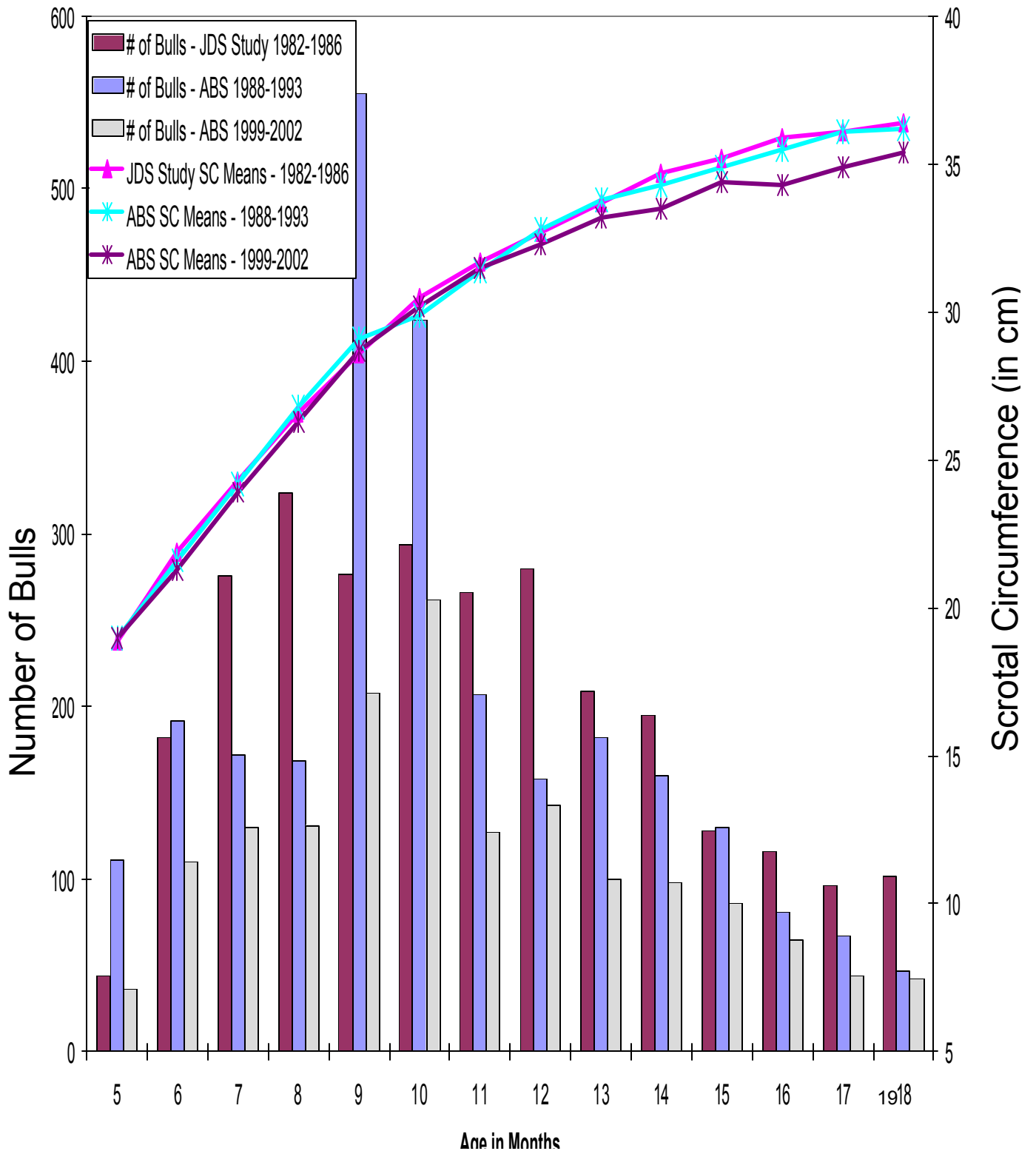
T=Table 5: 49 day non-return rate for cows inseminated with either optimum or sub-optimum number of fresh or frozen spermatozoa. (Adapted from Shannon & Vishwanath 1995) [30]

		49 Day Non-return Rate		
		Fresh	Frozen	Row Difference
<b>Number of Spermatozoa</b>	<b>Optimal<sup>a</sup></b>	<b>68.1%</b>	<b>67.6%</b>	<b>0.5%</b>
		<b>(n=14792)</b>	<b>(n=6004)</b>	
	<b>Sub-optimal<sup>b</sup></b>	<b>61.1%</b>	<b>59.7%</b>	<b>1.4%</b>
		<b>(n=9034)</b>	<b>(n=3710)</b>	
	<b>Column Difference</b>	<b>7.0%</b>	<b>7.9%</b>	

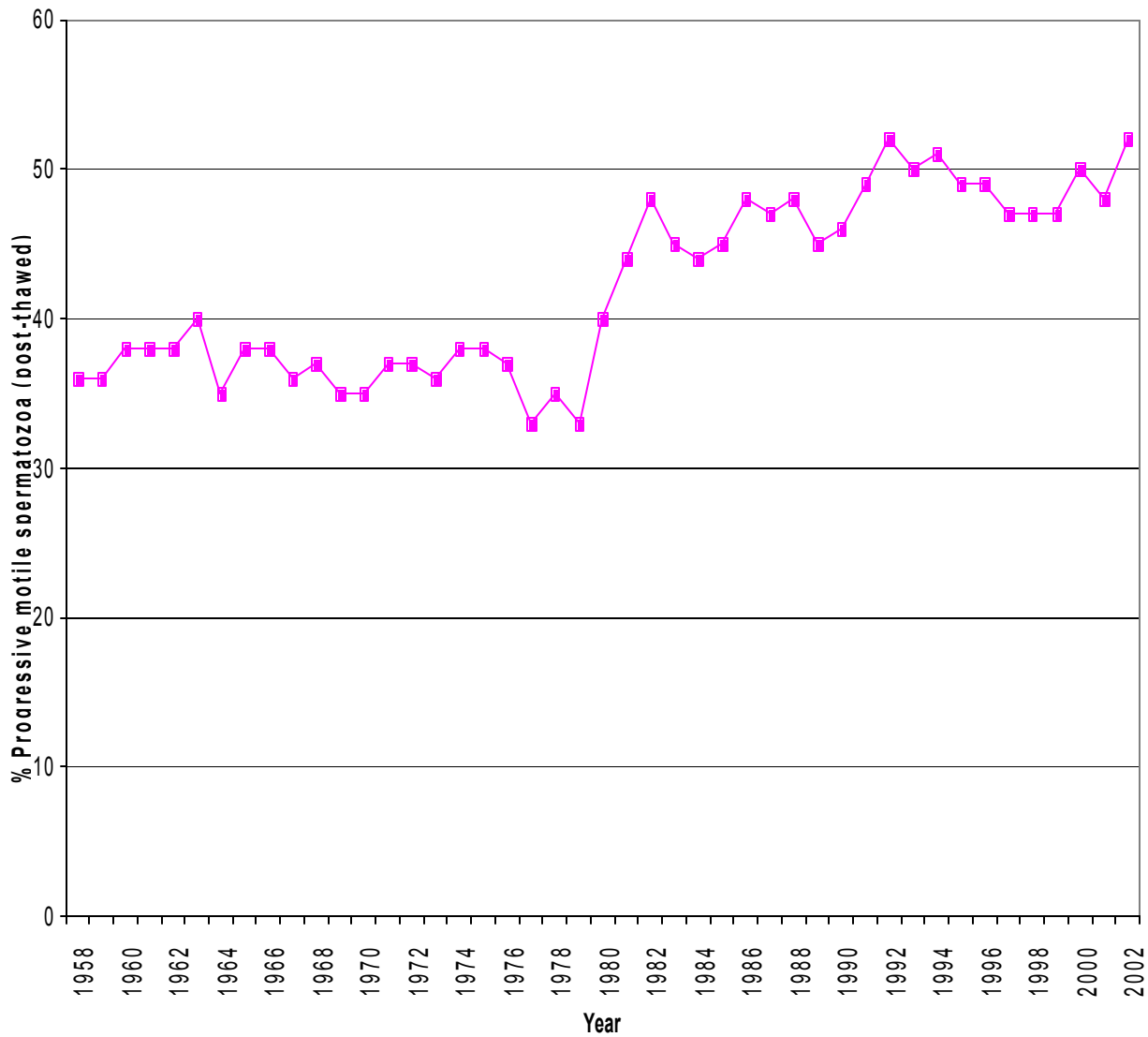
<sup>a</sup> =2x10<sup>6</sup> Fresh & 8x10<sup>6</sup> Frozen motile spermatozoa inseminated

<sup>b</sup> =0.4x10<sup>6</sup> Fresh & 2x10<sup>6</sup> Frozen motile spermatozoa inseminated

Figure 1. Mean scrotal circumference (SC) for bulls during three different time periods.



**Figure 2. Percent progressively motile spermatozoa (post-thawed) from all Holstein bulls  $\geq 4.5$  years old that were collected at ABS Global (ABS unpublished data).**



**Figure 3. Percentage of spermatozoa with a primary morphological abnormalities and percent total morphologically abnormal spermatozoa from all Holstein bulls  $\geq 4.5$  years old that were collected at ABS Global (ABS unpublished data).**

