Proceedings, Applied Reproductive Strategies in Beef Cattle
September 11 and 12, 2007, Billings, Montana

INSEMINATION RELATED FACTORS AFFECTING FERTILIZATION IN ESTROUS-SYNCHRONIZED CATTLE

R.G. Saacke, Professor Emeritus
Department of Dairy Science, Virginia Tech
Blacksburg, VA 24061

Introduction

In addition to the requirements for healthy well-managed cattle and the sound application of synchronizing drugs, many other factors can also play a role in determining the success of an AI–estrous synchronization program. Considering the economic investment in semen and drugs, the success of such a program must be judged on the basis of pregnancy rate to the first artificial insemination service. Also, a good first service pregnancy rate response usually signifies conditions are good for second service and the breeding season in general. Additional key factors to be considered as impacting pregnancy rate to first service are semen quality (primarily dependent on choice of bull), the timing of insemination and the competence of the inseminators in handling and placement of semen. In most breeding strategies, whether estrous synchronization is employed or not, the semen quality, placement, and timing of insemination are critical to a successful pregnancy. The nature of subfertility due to the male/inseminate is proving as complex as that due to the female. Research in our laboratory utilizing accessory sperm (measure of sperm available for fertilization) and subsequent embryo quality (measure of fertilizing sperm and egg competence) have given us some insights to the problems associated with attempts to optimize pregnancy rate to AI. In this presentation I would like to address some of these insights particularly those associated with the semen/bull and the timing of insemination.

Compensable and Uncompensable Seminal Deficiencies

We now know success or failure of an AI dose due to the male or inseminate resides in whether or not the egg was fertilized (fertilization rate) or whether or not the embryo developed normally and hatched in time to signal pregnancy to the dam (embryonic death). Both scenarios are embraced by semen quality and quantity and they must be considered together to address “pregnancy rate”. Salisbury and VanDemark (1961) were the first to suggest the nature of the relationship between sperm quality and quantity. They proposed that fertility increases with increasing numbers of viable sperm delivered to the cow up to a threshold, after which limiting factors in the female population become important and further increases in sperm are without effect on fertility. From the standpoint of semen quality, Pace et al. (1981) found this relationship to hold true for numbers of structurally intact and motile sperm in the inseminate. Sullivan and Elliott (1968) showed the minimum number of motile sperm required for maximum fertility (threshold) differed among bulls and bulls also differed in the maximum fertility at any

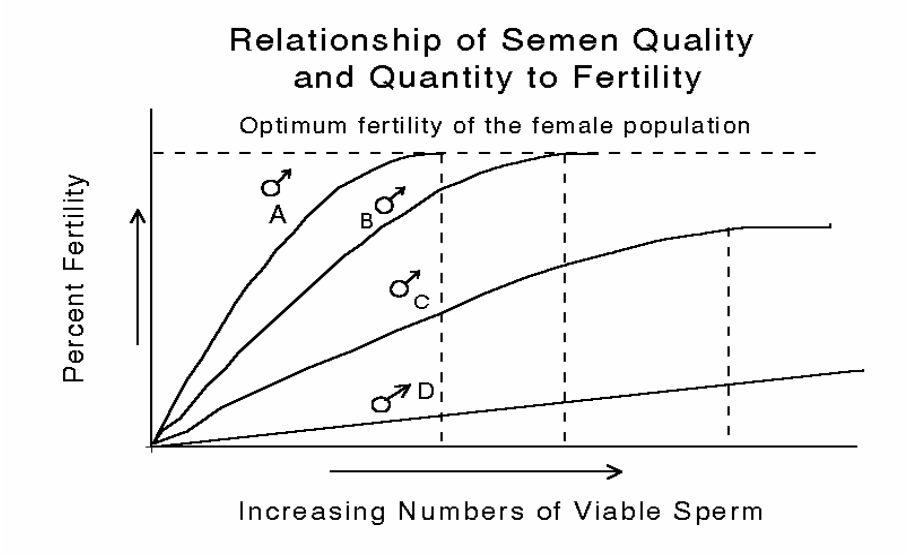

Figure 1. Relationship between pregnancy rate and the number of spermatozoa inseminated. The semen of different bulls varies in the maximum non-return rate and in the rate at which the maximum fertility is achieved with increasing sperm dosage (modified from Sullivan and Elliott (1968).

dosage (Figure 1). They also observed that low fertility bulls required more sperm be inseminated than high fertility bulls in order to reach their respective maximum fertility. They postulated the requirement of more sperm by the subfertile bulls was due to the presence of abnormal sperm unable to negotiate barriers in the female tract precluding their access to the site of fertilization. This was shown to be true in a later study (Saacke et al., 1998) where sperm with classically misshapen heads do not appear as accessory sperm in eggs following artificial insemination. From AI data in the Netherlands, den Daas et al. (1992) found the minimum number of sperm required to reach maximum fertility for a given bull (threshold) was independent of the maximum fertility achievable by that bull. Collectively, these studies, cited above, indicate it is now critical to recognize that seminal deficiencies fall into two major categories (compensable and uncompensable). Seminal deficiencies that are **compensable** would be those impacting pregnancy rates when numbers of sperm in the dosage are below threshold levels; i.e. pregnancy rate differences among bulls due to compensable seminal deficiencies would be minimized or eliminated simply by raising sperm numbers per AI dose (bull A vs B, Figure 1). Such adjustments in the AI dose are made by reliable AI organizations when such deficiencies are known and are recognized by many tests of sperm viability (motility, membrane integrity and other vital signs of cell life). Clearly, there are other compensable deficiencies still unknown to us and believed to be associated with properties of the sperm surface. However, where semen handling techniques or AI placement of semen is not adequate, impairment of pregnancy rate can be expected simply because lower than threshold numbers of viable competent sperm may be delivered to the cow. Seminal deficiencies that are **uncompensable** would be those that result in subfertility to AI or natural service regardless of sperm dosage and are represented by incompetent sperm that can fertilize, but not sustain an embryo. Such a deficiency is not compensable because incompetent sperm can preempt fertilization by a

competent sperm to the level that such incompetent sperm exist in the semen dose (bull C and D vs A and B, Figure 1). These deficiencies are intrinsic to the bull and can therefore only be minimized by bull evaluation and selection.

There is now good evidence many sperm with normal motility and morphology present in abnormal ejaculates are able to access the egg, but not competent to complete fertilization or sustain embryogenesis once these events are initiated (Barth, 1992; Courot and Colas, 1986; DeJarnette et al., 1992; Orgebin-Crist and Jahad, 1977; Setchell et al, 1988). Differences among bulls in embryonic development of their conceptuses have been reported at the time of routine recovery for embryo transfer (Miller et al., 1982) and after observation of embryo survival in recipients (Coleman et al., 1987). Bulls were also shown to differ in the development of their embryos following in vitro fertilization (Eyestone and First, 1989: Hillery et al., 1990; Shi et al., 1990; Eid et al., 1994). In low fertility bulls, early cleavage rates were reduced and pronuclear formation was delayed or impaired (Eid et al., 1994; Walters et al., 2006). Thus, incompetence in morphologically normal or near-normal spermatozoa of abnormal ejaculates appear to be the cause of the uncompensable component.

It should be recognized sperm with microscopically normal morphology but defective chromatin has been implicated in cases of male subfertility for some time (Gledhill, 1970). The chromatin structure assay developed by Evenson et al. (1980) revealed a strong positive association between heterospermic fertility in bulls (based upon genetic markers at birth) and stability of the sperm DNA to acid denaturation (Ballachey et al., 1988). Using this same assay, Karabinus et al., (1997) have shown sperm ejaculated before a mild thermal insult of the testis by scrotal insulation have more stable DNA than those ejaculated after scrotal insulation where abnormal sperm are also evident (Vogler et al., 1993). Acevedo et al. (2002) modified the chromatin structure assay such that sperm DNA stability to acid denaturation could be evaluated on the same sperm as judged morphologically. Applying this modification to the semen of scrotally insulated bulls, they reported spermatogenic disturbance caused by elevated testicular temperature resulted in the production of abnormal sperm and that vulnerability of sperm DNA to acid denaturation was positively associated with abnormal shaped sperm, but also extended to normal shaped sperm in the abnormal samples. This tends to confirm occurrence of morphologically abnormal sperm can signal chromatin abnormalities and potential incompetence among both normal and abnormally appearing sperm in the same sample. It also underlines the fact while female sperm selection appears amazingly strong based upon sperm shape and motility (Saacke et al., 1998), it is far from absolute in excluding incompetent sperm from accessing the egg.

Although it is important we recognize both the compensable as well as the uncompensable seminal traits in our breeding-male populations, it is clear we must focus most seriously on the uncompensable traits since these result in subfertility regardless of sperm numbers in the inseminate, AI technique or reproductive strategy applied. Bulls having uncompensable deficiencies in their semen should be eliminated from use in our herds wherever possible. At our current state of knowledge, such bulls are best avoided by using AI bulls from reliable sources where semen morphology is a routine part of the evaluation process or in the case of natural bulls where semen morphology is a strong part of the BSE exam.

Accessory Sperm and Their Implication to Pregnancy Rate

A research area giving us insight to semen related problems involves accessory sperm. Accessory sperm are those sperm trapped in the zona pellucida (outer covering of the egg), one of the important egg vestments sperm must penetrate in order to fertilize. Although there is only one fertilizing sperm, a range in number of sperm may be simultaneously competing for this honor. Once the fertilizing sperm enters the egg proper, a reaction occurs stopping progress of these competing sperm as well as the binding of additional sperm to the surface of the zona pellucida. Thus, accessory sperm are thought to represent, in number and quality, those sperm competing for fertilization in the oviduct of the cow during that short window in time provided by the ovulated fertilizable egg.

Through several years of experimentation in our lab we have recovered nearly 1000 eggs/embryos from single-ovulating cows 6 days post artificial insemination (nearly 30 different bulls were represented in these studies). Figure 2 shows the distribution of accessory sperm found in the zona pellucida of embryos and eggs from these cows as being very skewed, having an average, median and mode of 12.0, 2.4 and 0 sperm per ovum/embryo, respectively. This signifies that Nature intends that only a few sperm compete for fertilization at a given time. Of reproductive interest is the association of accessory sperm number per egg/embryo to the fertilization status and embryo quality. This is best described by the median number (50 percentile of cows) of accessory sperm per egg/embryo (Table 1). Clearly, unfertilized eggs are simply sperm hungry, having a median accessory sperm number of 0. These data also show embryo quality tends to be positively related to median accessory sperm number. Good to excellent embryos have a higher accessory sperm number than do degenerate or fair to poor embryos; but the mode remains 0 regardless of embryo quality. This rather small difference in median sperm has been interpreted to suggest that the larger accessory sperm numbers are most likely associated with higher embryo quality because they represent greater competition among potential fertilizing sperm at the time of fertilization. There is evidence this competition favors a more competent sperm (i.e., sperm selection may also occur at the zona pellucida of the egg, Howard et. al., 1993) as well as at other locations in the female tract (previously reviewed, Saacke et al., 2000). On this basis, we ascribed a score to the embryos within categories of increasing accessory sperm number to determine the approximate number of accessory sperm (competing sperm) required to maximize embryo quality in artificially inseminated cows. These data are presented in Figure 3 and were based upon 804 embryos recovered from the 927 ova/embryos represented in Figure 2 and Table 1. It is apparent from Figure 3 nearly 10 sperm per embryo were necessary to reach the maximum embryo quality index, after which increasing accessory sperm numbers had no further influence on embryo quality. Regardless of embryo quality, it should be remembered the mode in accessory sperm number remained 0, i.e., the most common occurrence was one sperm per egg, the fertilizing sperm, again suggesting Nature intended very few sperm approach the egg at any one point in time. On the other hand, this exercise stresses the importance of semen handling and placement in the cow if we are to achieve threshold or above threshold numbers of sperm to the egg (i.e., approach 10 sperm/egg) necessary to maximize both fertilization rate and embryo quality for a general population of bulls.

It should be clear the large variation in accessory sperm (expressed as SD) within and across fertilization/embryo status categories (Table 1) would preclude any use of accessory sperm numbers in predicting male fertility, particularly at numbers of observations (eggs) practical using this approach. This large variation underlines the fact we still have much to learn about conditions optimizing fertility in cattle. Nevertheless, increasing accessory sperm numbers across a sufficient number of inseminations could indicate directions to be taken in adopting reproductive practices and strategies favoring improved pregnancy rates.

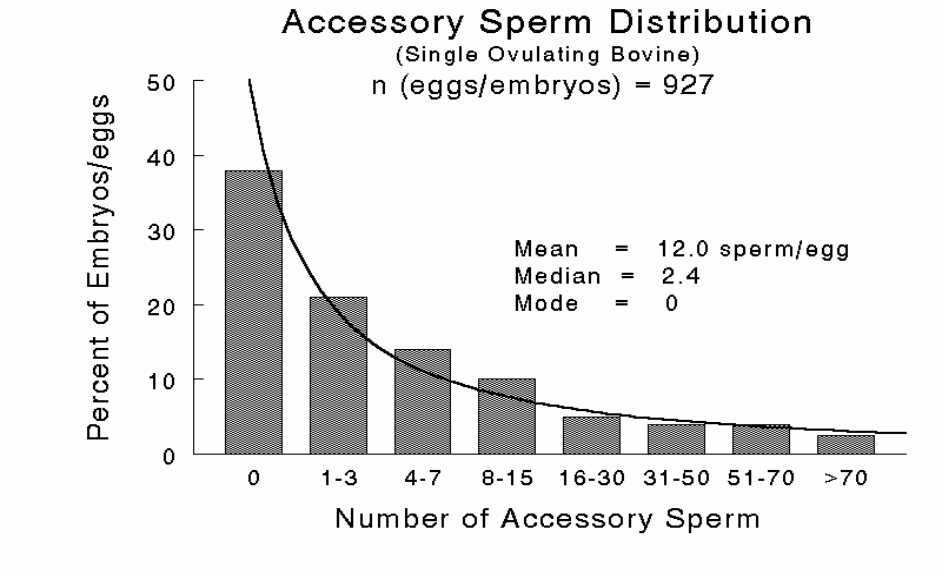

Figure 2. Frequency distribution of accessory sperm per embryo or ovum in artificially inseminated single-ovulating cows. Quality and quantity of semen used varied, but was within acceptable standards for commercial artificial insemination. Similar distributions have been reported for individual experiments utilizing both frozen and fresh semen (Saacke et al., 2000).

Table 1. Relationship of accessory sperm per embryo/ovum to fertilization status and embryo quality (n=927)

Fertilization status/ Embryo quality	n	Mean ± SD	Median
Excellent/good	449	24.5 ± 44.1	**7**
Fair/poor	213	17.2 ± 32.2	**5**
Degenerate	80	13.5 ± 38.1	**1**
Deg/UFO	12	2.7 ± 5.7	**0.5**
Unfertilized	173	1.6 ± 16.5	**0**

Embryo quality based upon Lindner and Wright, 1983 as modified for degenerate embryos by DeJarnette, et al., 1992

It is important one understands how embryo quality (as judged) affects pregnancy rate, particularly in assessing uncompensable semen traits. The best data on this point is Lindner and Wright (1983), who developed the embryo scoring system used in the data presented above. They showed embryos classified as excellent to good produce twice as many pregnancies upon transfer to recipients as those classified fair to poor. One would expect much of this difference in embryo performance to carry over to embryos permitted to remain in utero. Of course degenerate embryos and unfertilized eggs produce no pregnancies under any circumstance. Based upon the median number of 2.4 accessory sperm per egg/embryo (Figure 2) and the threshold requirement of nearly 10 sperm per ovum/embryo to optimize embryo quality (Figure 3), efforts to raise accessory sperm number have been undertaken and previously reviewed (Saacke et al., 1994 and 2000). These efforts will not be repeated here except to emphasize two of the major positive factors impacting accessory sperm numbers per egg/embryo relevant to estrous synchronization and timed insemination, namely, choice of bull and time of insemination.

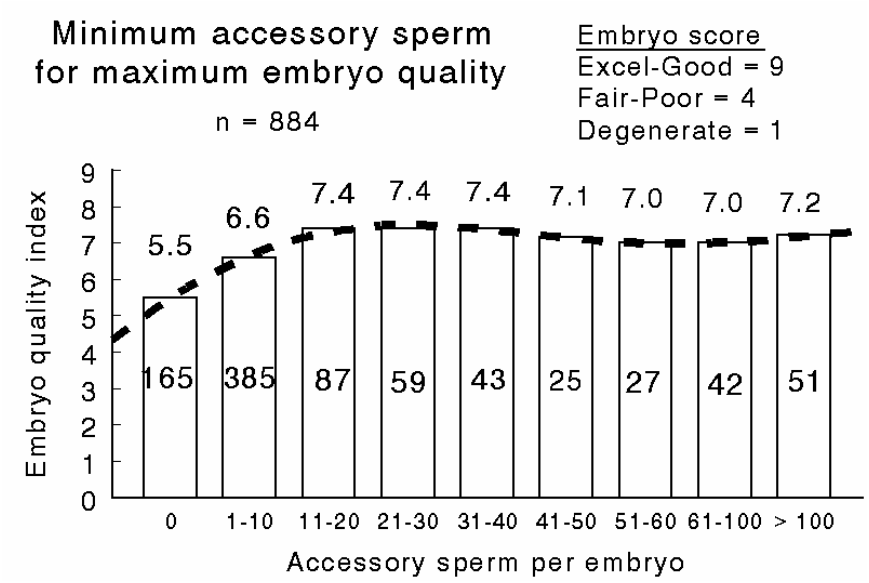

Figure 3. Histogram showing the numbers of accessory sperm required to maximize embryo quality index for 6 day-old embryos (morulae) derived from artificial insemination of single-ovulating cows. Embryo grading was according to Lindner and Wright (1983) as modified by DeJarnette et al., (1992). Embryo quality index was the average embryo quality based on the numerical score listed above. As may be noted, a minimum of approximately 10 accessory sperm per embryo was required to maximize embryo quality index. The number within each bar is the number of embryos recovered in that accessory sperm category.

The Effect of Bulls and Time of Insemination on Sperm Access to the Egg and Embryo Quality

Even when cows are bred at the conventional time following onset of heat (approximately 6-16 hours following onset), there is considerable variation among bulls with respect to numbers of sperm accessing the egg (Nadir et al., 1993). Data from this study comparing four bulls is presented in Table 2. Clearly, Bull A in this comparison has high egg access as denoted by the high accessory sperm number (median of 40 sperm per egg) compared to the other three bulls. It would be expected such a bull as A would perform as well at low sperm dosages as at normal dosage and/or this bull would be less vulnerable to inseminator error in semen placement and handling than would other bulls. Such a bull would be considered to have little to no compensable deficiencies and easily meet threshold numbers of sperm to the cow by AI. Under the same premise, bulls B and C would also match the fertility and embryo quality of bull A, but one would expect that while sperm dosage is appropriate, there is less room for inseminator or semen handling error with these two bulls. For bulls B and C, pregnancy rates could be expected to depend more heavily on dilution rates, inseminator competence and timing of insemination. Based on a median of two sperm per egg, bull D might be more marginal in optimizing fertilization rate and embryo quality under current use in AI. The seminal differences we are addressing across these four bulls would be considered compensable differences. Some of the semen traits involved in these differences are known and used by AI organizations in processing semen and determining sperm dosage rate. However, as pointed out earlier, there are compensable differences among bulls we still do not understand and can only determine by fertility data from artificial insemination of adequate numbers of cattle at known semen dosages.

Table 2. Accessory sperm differences per embryo/egg among bulls used at the same insemination dosage

Bull	n	Median	Mean ± SD
A	25	40	53 ± 61
B	37	8	15 ± 23
C	16	13	36 ± 65
D	20	2	11 ± 16

Nadir et al., 1993

With respect to differences among bulls important to embryo quality, i.e., the competence of a bull's fertilizing sperm or the uncompensable deficiency in his semen; our best judge of this is the occurrence of abnormal sperm in the semen, as pointed out earlier in this paper. Abnormal sperm in the semen reflect the health of the spermatogenic process in the testes of the bull and in particular, the health of the DNA contributed to the embryo by the male. DeJarnette et al., (1992) examined the 6-day-old embryos from cows bred to semen of AI bulls having average and below average quality (within the AI center) based upon counts of abnormal sperm. Their data is shown in Figure 4. Clearly, the below average semen produced fewer excellent to good embryos and greater numbers of degenerate embryos and unfertilized eggs when compared to semen of average quality,

again emphasizing the importance of uncompensable seminal traits to pregnancy rate. Today, bulls in AI are more rigorously screened for numbers of abnormal sperm prior to acceptance into AI. In addition, in reliable AI organizations, routine examination of semen for abnormal sperm is practiced to check for changes in a bull's spermatogenic status. Sperm morphology evaluation is also one of the main components of the BSE (breeding soundness exam) of bulls practiced by veterinarians in approving breeding bulls for service. Still posing a particular problem in uncompensable semen deficiencies among beef breeds, are fat bulls and a percentage of those coming off "hot rations" from test stations, where testicular thermoregulation has been impaired by excessive inguinal fat (Kastelic et al. 1996). Again, abnormal sperm morphology is the most prominent method of recognizing the consequences of abnormal testicular thermoregulation in such bulls.

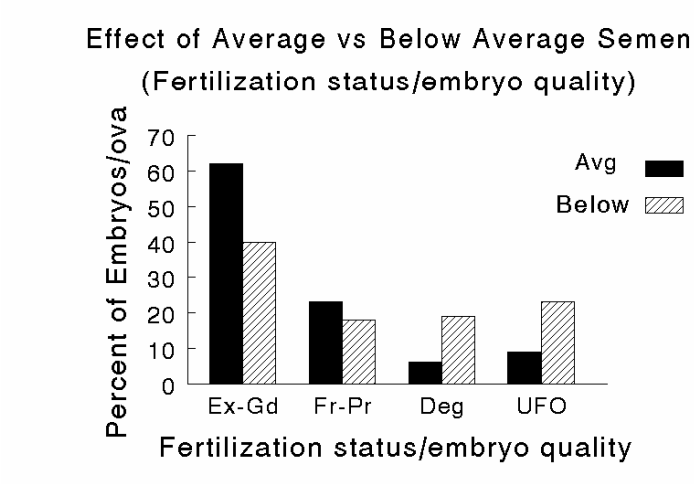

Figure 4. Effect of average and below average semen (based upon content of abnormal sperm) on fertilization status/embryo quality in single ovulating cattle. Both, fertility and embryo quality were influenced by the semen as noted in the shift in distribution across categories (n = 21 and 22 for the average and below average semen, respectively). (DeJarnette et al. 1992).

The effect of insemination time on numbers of accessory sperm, fertilization status and embryo quality has been studied by Dalton et al., 2001. In this experiment, the HeatWatch® system was used to dictate time of artificial insemination for each cow. In this heat detection system, an electronic device is placed on the rump of the cow and a signal transmitted via antennas to a computer when the device is activated for 2 seconds by pressure of a mounting cow. On this basis, first mount, duration of mounting and number of mounts were permanently recorded along with the identification of the standing cow. In lactating Holsteins, ovulation occurs 27.6 ± 5.4 hours following the first mount for either natural estrous cycles or prostaglandin synchronized cycles (Walker et al., 1996). Experimental artificial insemination time was 0 hour, (heat onset indicated by first mount), 12 or 24 following first mount. However, due to logistics associated with monitoring the computer every three hours followed by retrieving the cow for insemination, actual times of insemination were: 2.0 ± 0.9 hours, 12.1 ± 0.6 and 24.2 ± 0.7 hours following the first mount, respectively. Six days following insemination, the embryo was recovered non-surgically and examined for fertilization status/embryo

quality and numbers of accessory sperm according to previously published methods (DeJarnette et al. 1992). Artificial insemination was to one of three bulls used at random and balanced in number of resulting eggs/embryos recovered for each time of insemination. Accessory sperm data are presented in Table 3. Clearly, accessory sperm number per embryo/egg was favored by breeding later, rather than earlier. Fertilization rate and embryo quality are presented in Figure 5 for each insemination interval (0, 12, or 24 hours post estrus onset). From Figure 5, increasing fertilization rate can be observed to follow increasing accessory sperm number (Table 3), as expected. Fertilization rate is favored by breeding late (24 hours post heat onset) and poorest by breeding early, near onset of heat. However, examination of embryo quality in relation to time of insemination shows a shift from high quality embryos achieved by inseminations at/near onset of heat to low quality embryos from insemination at 24 hours following heat onset. On the basis of these data it appears optimum reproductive efficiency (pregnancy rate) is a compromise using our current techniques and recommendations in AI. If we inseminate too early, we suffer from lower fertilization rates (but embryo quality is good) and if we breed too late, we suffer from lower embryo quality (but our fertilization rate is good). Thus, the intermediate time of 12 hours post heat onset would prove optimal when using a precise method for determining heat onset (Figure 6). This optimum was verified in field studies using "HeatWatch®" (Dransfield et al. 1998) where 6-16 hours post onset of heat provided the best pregnancy rates. The basis for pregnancy rate failure by breeding late (24 hour post onset) could reside in the fact that we would often have an aging egg waiting for sperm if we assume ovulation occurs 27.6 ± 5.4 hours post heat onset as detected by HeatWatch®. Sustained sperm transport to the site of fertilization in the oviduct requires a minimum of 4-6 hours following insemination in the cow (Hunter and Wilmut, 1984). Thus, sperm arrival in the oviduct following a 24-hour insemination would be 28 to 30 hours post heat onset, after many eggs were already ovulated. In the current study, this would indicate that a rather large portion of eggs would be aging awaiting sperm arrival. This undoubtedly attributes most of the degenerate embryos to late insemination (aging ovum) rather than a male-related uncompensable trait. On the other hand, the high embryo quality associated with early insemination suggests that duration of sperm residence in the female tract may result in exertion of additional selection pressure favoring fertilization by a more competent sperm, particularly where there are uncompensable sperm deficiencies in the semen (Figure 6). The correct explanation is probably a combination of the two but must await further research.

Table 3. Effect of artificial insemination time on accessory sperm per embryo or egg

(breeding time post onset of estrus based on HeatWatch System®)

Treatment	n	Mean ± SD	Median	% Fert
0 hour	39	9 ± 23	1	66
12 hour	39	21 ± 46	2	74
24 hour	39	33 ± 53	4	82

- Ovulation 27. 6 ± 5.4 hours
- 25 x 10^6 sperm/dose

(Dalton et al., 2001)

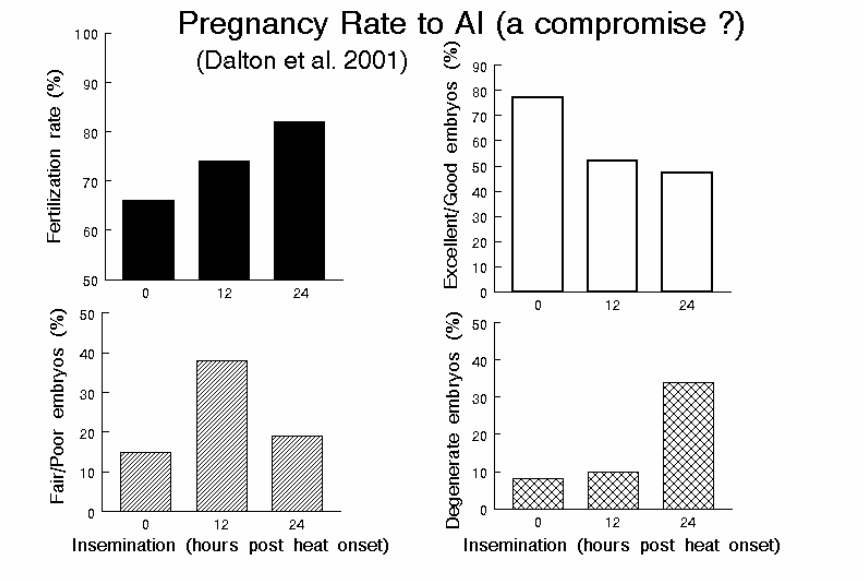

Figure 5. Effect of time of artificial insemination following onset of standing heat (Heat Watch System®) on fertilization status and embryo quality judged 6 days following artificial insemination (n = 117). (Dalton et al. 2001).

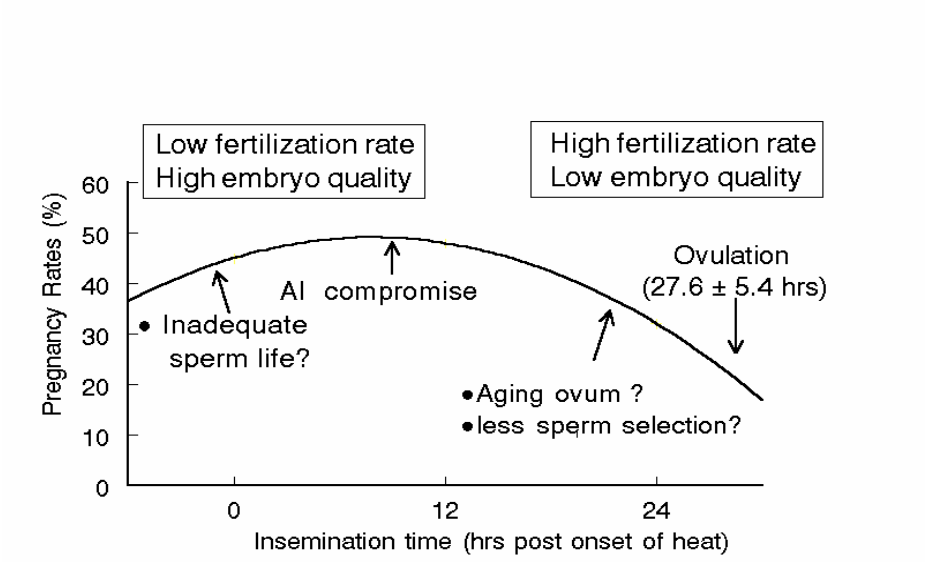

Figure 6. Calculated pregnancy rate from data presented in Figure 5 and based upon the ability of embryos classified excellent to degenerate to constitute a pregnancy (according to Lindner and Wright, 1983). AI as a compromise is based upon early inseminations being inadequate due to high levels of unfertilized ova, and late inseminations characterized by poor embryo quality, most likely due to an aging egg. However, high embryo quality appears to be associated with early insemination and high fertilization rates are associated with late insemination (Saacke et al., 2000).

Closing Comments

Important to the insemination strategies employed with the new burgeoning regimes of estrous synchronization is knowing the time of ovulation and the variation in time over which ovulation can be expected in a group of treated animals. Only by such information can we make the correct decision on when to inseminate in relation to injection events or behavioral clues predicting ovulation. The data presented here would indicate insemination must be late enough to maximize sperm access to the freshly ovulated egg, but not so late to ignore sperm transport time in the cow and risk the possibility of an aging egg awaiting sperm arrival in the cow's oviduct. Thus, in contrast to the study reported with ovulation at 27 hours post heat onset, if a synchronization regime were to postpone ovulation until 30 or 35 hours following heat onset, the 24-hour insemination could be the best in optimizing pregnancy rate (both fertilization rate and embryo quality). Clearly, the CL and follicular control of the estrous cycle in cattle, currently under intensive research, offers tremendous advantages in synchronizing ovulation and tightening the variation in time of ovulation.

Finally, I would end this discussion by again recognizing the potential magnitude of bull differences that can be encountered in a synchronization program. Differences observed among the three bulls in response to time of insemination for the study reported by Dalton et al., 2001 in Table 3 are shown in Figure 7. Although the trends were similar, the magnitude of differences in performance of bulls at different insemination times was quite great. In a timed insemination program, Bull A would be considered to perform well over a broad time span relative to ovulation time, whereas bulls B and C really required later breeding to optimize their efficiency in sperm access to the egg. Unfortunately, and as you might expect, this is difficult, time consuming, expensive data to acquire and therefore not available on commercial bulls. The best protection one can have is to be aware of bull differences and know the expected time and variation in ovulation in order to choose an insemination time maximizing results to most bulls. Lastly, subscribing to a reputable semen source is the best protection against the use of poor quality semen.

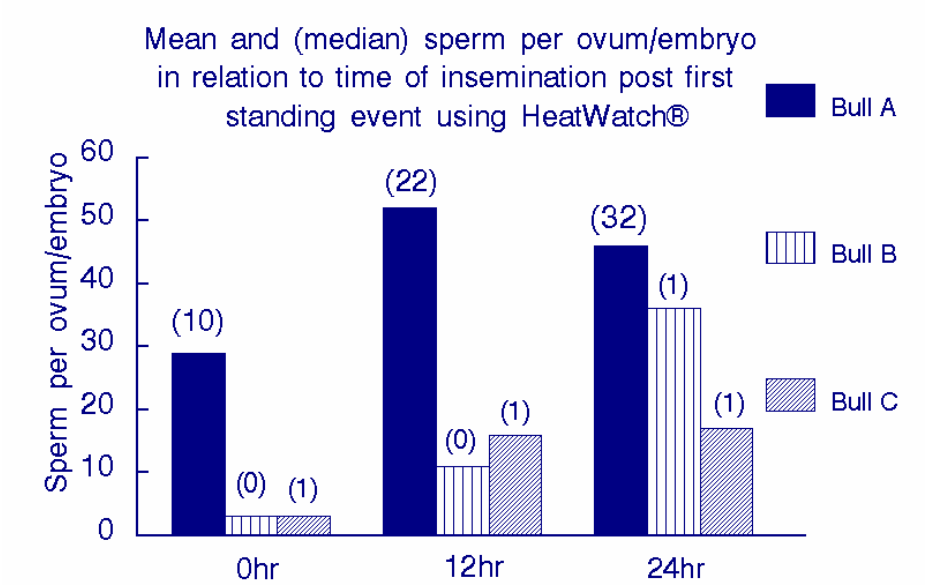

Figure 7. Variation among bulls in sperm access to the egg relative to time of insemination post heat onset. Mean sperm per egg/embryo is shown by the bars and the median number in brackets. Bull A has adequate numbers of sperm accessing the egg at all breeding times while bulls B and C require insemination closer to ovulation (Dalton et al., 2001).

Acknowledgements

I am very appreciative of the support of the following agencies relative to our male research regarding accessory sperm, fertilization and embryo quality in artificially inseminated cattle: Select Sires Inc., National Association of Animal Breeders and the Virginia Ag Council.

Literature Cited

Acevedo, N, JH Bame, LA Kuehn, WD Hoenboken, DP Evenson, RG Saacke. Sperm chromatin structure assay (SCSA) and sperm morphology. In: Proceedings of the fifteenth Tech Conference on Artificial Insemin Reprod; p 84-90. 2002.

Ballachey, BE, DP Evenson, RG Saacke. The sperm chromatin structure assay: relatonship with alternate tests of semen quality and heterospermic performance of bulls. J. Andrology 9:109-115. 1988.

Barth, AD. The relationship between sperm abnormalities and fertility. In Proc. 14th Tech. Conf. on Artif. Insem. and Reprod., Nat'l Assoc. Animal Breeders, Columbia, MO pp. 47-63. 1992.

Coleman, DA, RE Dailey, RE Leffel, RD Baker. Estrous synchronization and establishment of pregnancy in bovine embryo transfer recipients. J. Dairy Sci. 70:858-866. 1987.

Courot, M, G Colas. The role of the male in embryonic mortality (cattle and sheep). In: Embryonic Mortality in Farm Animals, M Greenan and MG Diskin (Editors), Dordrecht, Martinus Nijoff (Publishers) pp 95-203. 1986.

Dalton, JC, S Nadir, JH Bame, M Noftsinger, RL Nebel, RG, Saacke. Effect of time of insemination on number of accessory sperm, fertilization rate, and embryo quality in nonlactating dairy cattle. J. Dairy Sci. 84:2413-2418. 2001.

DeJarnette, JM, RG Saacke, J Bame, CJ Vogler. Accessory sperm: their importance to fertility and embryo quality, and attempts to alter their numbers in artificially inseminated cattle. J Anim Sci 70:484-491. 1992.

den Daas, N. Laboratory assessment of semen characteristics. Anim Reprod Sci, 28:87-94. 1992.

Dransfield, MGB, RL Nebel, RE Pearson and LD Warnick. Timing of insemination for dairy cows identified in estrus by a radiotelemetric estrus detection system. J. Dairy Sci., 81:1874-1882. 1998.

Eid, LN, SP Lorton, JJ Parrish. Paternal influence on S-phase in the first cell cycle of the bovine embryo. Biol. Reprod. 51:1232-1237. 1994.

Evenson, DP, Z Darznikiewicz, MR Melamed. Relation of mammalian sperm chromatin heterogeneity to fertility. Science 240:1131-1134. 1980.

Eyestone, WH, NL First. Variation in bovine embryo development in vitro due to bulls. Theriogenology 31: 191-196. 1989.

Gledhill, BL. Enigma of spermatozoal DNA and male infertility: a review. Am. J. Vet. Res. 31:539-549. 1970.

Hillery, JL, JJ Parrish, NL First. Bull specific effect on fertilization and embryo development in vitro. Theriogenology 33:249-252. 1990.

Howard, JG, AM Donoghue, LA Johnston, DE Wildt. Zona pellucida filtration of structurally abnormal spermatozoa and reduced fertilization in teratospermic cats. Biol Reprod 49:131-139. 1993.

Hunter, RHF, I Wilmut. Sperm transport in the cow: periovulatory redistribution of viable cells within the oviduct. Reprod Nutr Develop 24:597-603. 1984.

Karabinus, D, CJ Vogler, RG Saacke, DP Evenson. Chromatin structural changes in bovine sperm after scrotal insulation of Holstein bulls. J. Androl. 18:549-555. 1997.

Kastelic, JP, RB Cook, GH Coulter, RG Saacke. Insulating the scrotal neck affects semen quality and scrotal/testicular temperatures in the bull. Theriogenology 45:935-942. 1996.

Lindner, GM, RO Wright Jr. Bovine embryo morphology and evaluation. Theriogenology 20:407-411. 1983.

Miller, D, M Hrudka, WF Cates, R. Mapletopft. Infertility in a bull with a nuclear sperm defect: a case report. Theriogenology 17:611-621. 1982,

Nadir, S, RG Saacke, J Bame, J Mullins, S Degelos. Effect of freezing semen and dosage of sperm on number of acessory sperm, fertility and embryo quality in artificially inseminated cattle. J Anim Sci 71:199-204. 1993.

Orgebin-Crist, M, C Jahad. Delayed cleavage of rabbit ova after fertilization by young epididymal spermatozoa. Biol. Reprod. 16:358-363. 1977.

Pace, MM, JJ Sullivan, FI Elliott, EF Graham, GH Coulter. Effects of thawing temperature, number of spermatozoa and spermatozoal quality on fertility of

bovine spermatozoa packaged in 0.5-ml french straws. J Anim Sci 53:693-701. 1981.
Saacke, RG, JM DeJarnette, J Bame, DS Karabinus and SS Whitman. Can spermatozoa with abnormal heads gain access to the ovum in artificially inseminated super- and single-ovulating cattle? Theriogenology 50:117-128. 1998.
Saacke, RG, S Nadir, RL Nebel. Relationship of semen quality to sperm transport, fertilization, and embryo quality in ruminants. Theriogenology 41:45-50. 1994.
Saacke, RG, JC Dalton, S Nadir, RL Nebel and JH Bame. Relationship of seminal traits and insemination time to fertilization rate and embryo quality. Anim. Reprod. Sci., 60-61:663-677. 2000.
Salisbury, GW, NL Vandemark. Physiology of reproduction and artificial insemination of cattle. San Francisco, Freeman, 1961. p361.
Setchell, BP, MJ Occhio, MS Hall, MS Lourie, MJ Tucker, JLZupp. Is embryonic mortality increased in normal female rats mated to subfertile males? J. Reprod. Fertil., 83:567-574. 1988.
Shi, KS, KH Lu, I. Gordon. Effect of bulls on fertilization of bovine oocytes and their subsequent development in vitro. Theriogenology 33:324-333. 1990.
Sullivan, JJ, FI Elliott. Bull fertility as affected by an interaction between motile spermatozoa concentration and fertility level in artificial insemination. VI Int'l Cong. Anim. Reprod. Artif. Insem. 2:1307. 1968.
Vogler, CJ, J Bame, JM DeJarnette, ML McGilliard, RG Saacke. Effects of elevated testicular temperature on morphology characteristics of ejaculated spermatozoa in the bovine. Theriogenology 40:1207-1219. 1993.
Walker, WL, RL Nebel, ML McGilliard. Time of ovulation relative to mounting activity in dairy cattle. J Dairy Sci 79:1555-1561. 1996.
Walters, AH, RG Saacke, RE Pearson, FC Gwazdauskas Assessment of pronuclear formation following in vitro fertilization with bovine spermatozoa obtained after thermal insulation of the testis. Theriogenology 65:1016-1028. 2006.