Assessing Bull Fertility

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Introduction

Our concepts involving assessment of bull fertility by evaluation of semen are changing rapidly. The overall research objective has been to predict the fertility of a male by subjecting the semen to appropriate laboratory/field testing. We have not met this objective. Many tests and combinations of tests were found to have significant correlations to fertility, but fell far short of being predictive of fertility. A better understanding of the interaction of the male / inseminate with the female tract and ovum appears critical to semen and male evaluation. In this presentation of assessing bull fertility, I would to like to summarize our work on accessory sperm and the ova / embryos from which they are taken and, wherever possible, relate the findings to our understanding of male / inseminate fertility. Hopefully, from such a base, we can formulate a philosophy toward the assessment of the reproductive capacity of the bull.

It is important first that we consider the rationale for pursuing this approach. Central to our current concepts in assessing male fertility or fertility of a semen dose used in artificial insemination is the relationship of semen quality to semen quantity. It is now clear that there are semen quality differences among males that are **compensable** in that fertility differences among such males can be minimized or eliminated by adjusting the quantity of sperm in the dosage (see 29 for a review). On the other hand, there are subfertile males that cannot be brought to normal fertility by increasing the inseminate dosage, thus rendering the semen traits or deficiencies of such males, **uncompensable**. These differences among males are illustrated in Figure 1. Den Dass[4] has recently shown that the rate at which bulls reach their maximum conception rate as semen dosage is increased varies and that this variation is unrelated to their maximum fertility (Figure 2). Thus, semen of a given male may contain compensable, uncompensable or both factors and each to a different degree. Therefore, to understand the nature of the impact that a given male/inseminate has on reproduction or to make progress toward improving fertility via the male/inseminate, we must approach the problem from the standpoint of differentiating the nature of the deficiency (or deficiencies). We must be able to identify sperm characteristics or deficiencies that

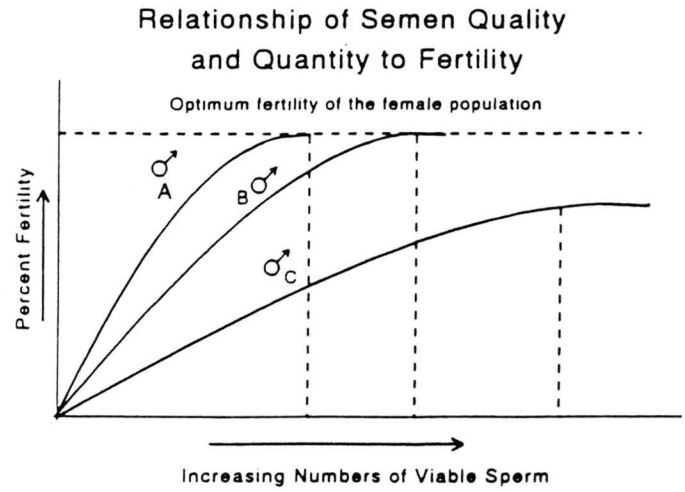

Figure 1. Bulls differ in the minimum number of viable sperm required for maximum conception (compensable factors in semen, difference between Bull A and B) and in the ultimate level of fertility of which they are capable (uncompensable factors in semen, if below the optimum fertility of the female population, Bull C). See 29, for a review.

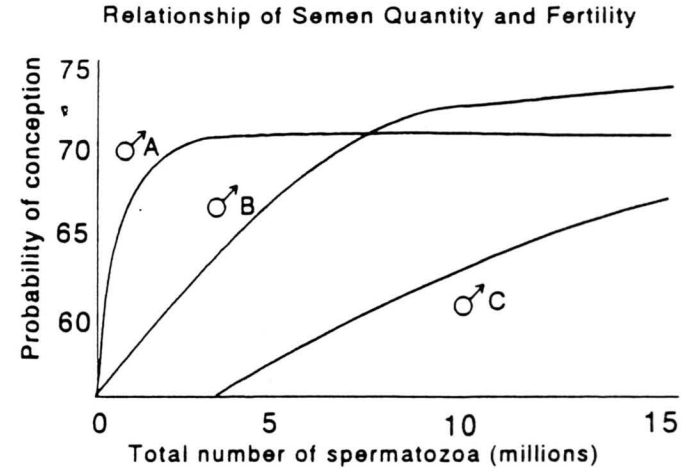

Figure 2. Relationship between non-return rate and the number of spermatozoa inseminated. The semen of different bulls varies in the maximum non-return rate and in the rate at which the maximum fertility is achieved with increasing sperm dosage. (adapted from den Dass, 4)

preclude availability of sperm for fertilization (compensable traits) and identify sperm traits or deficiencies associated with incompetent fertilizing sperm, i.e., those sperm that can initiate but not complete the fertilization process or sustain early embryogenesis (uncompensable traits).

In cattle, major barriers of sperm transport to the site of fertilization are presented in Figure 3 and have been reviewed.[26] The quantity of sperm reaching the site of fertilization is quite varied from experiment to experiment, but is also relatively small in relation to that of the inseminate.[14] A rich literature, important to evaluation of semen, is now accumulating to indicate that the quality of sperm that reach the oviductal isthmus and the ampullary-isthmus junction is enriched in both viability and normal morphology compared to that of the inseminate which we evaluate.[12, 23, 27] In addition, there is now evidence that the vestments of the ovum offer a final barrier(s) against participation, in fertilization, of sperm with certain traits.[8] It is clear that we must understand this entire "filtration system" in order to evaluate semen/males intelligently.

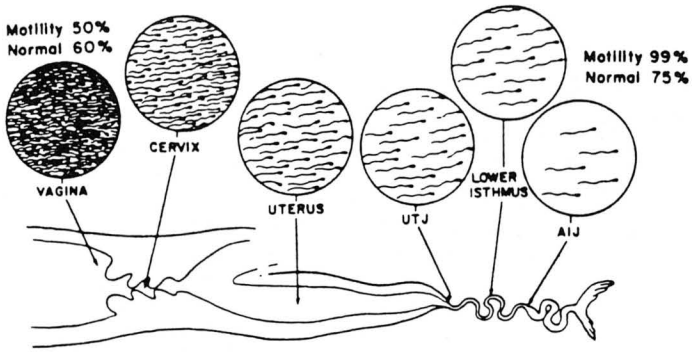

Figure 3. Schematic summarizing work in many laboratories regarding the major barriers to sperm transport in the female tract. Numbers of sperm reaching the egg at the AIJ (ampullary-isthmus junction) are relatively small in relation to those inseminated; however, they are enriched in quality, particularly viability and to a lesser extent, morphology (see 26 for a review).

The Accessory Sperm Approach

Accessory sperm are those sperm entrapped in the zona pellucida of the egg/embryo, one of the important vestments sperm must penetrate in order to fertilize (Figure 4). Although there is normally only one fertilizing sperm, a range in numbers of sperm may be simultaneously competing for this honor. Once the fertilizing sperm enters the cytoplasm of the ovum, a reaction occurs (called the "zona reaction", "cortical reaction", or "block to polyspermy", see 3, for a review of this process). This is an important process since more

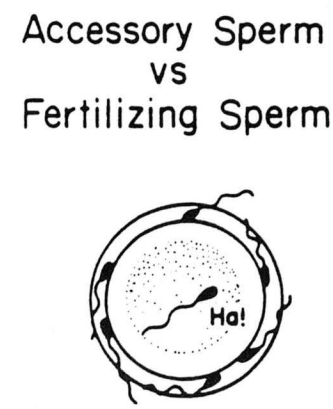

Figure 4. Accessory sperm are those sperm in the process of penetrating the egg at the time the fertilization process (entry of the fertilizing sperm) occurs. The egg reacts to fertilization by a process called the "zona reaction" which alters the zona pellucida such that sperm in the zona cannot continue progressing and new sperm can no longer attach. See 3, for a review of this process.

than one sperm fertilizing the egg would result in embryonic death. Therefore, accessory sperm represent sperm capable of traversing the barriers of the female reproductive tract, undergoing the functional sperm processes of capacitation, egg recognition and binding and the true acrosome reaction. Numbers of accessory sperm are thought to represent or parallel the numbers of potential fertilizing sperm available to the ovum during the time required for sperm penetration and fertilization. The duration required for trans zona migration of the fertilizing sperm under natural *in vivo* conditions is not known for many species, including cattle.[2] However, additional factors which could affect accessory sperm number include: 1. differences in penetrability of the zona due to natural differences among females, hormonal manipulation (superovulation), or in vitro ovum maturation, 2. differences in the speed of penetration by a fertilizing sperm, and, 3. differences in the speed of the zona reaction (block to polyspermy).

Our purpose in measuring the quantity and quality of accessory sperm as well as the associated fertilization status and embryo quality from which these sperm come is to better understand the potential impact of the male/inseminate on reproductive efficiency. Of particular interest is the desire to distinguish between the compensable and uncompensable traits in semen. Our quantitative and qualitative evaluation of accessory sperm and the embryo from which they come is determined 6 days following insemination. At this time the embryos/ova are flushed from the uterus nonsurgically, as they would be in embryo recovery destined for transfer. An embryo (expected to be in the morula stage at 6 days post insemination) is graded according to the scheme of Lindner and Wright[15] as either excel-

lent, good, fair, or poor. This evaluation considers the compactness and homogeneity of the cell mass. The relevance of this classification to reproductive efficiency is that, upon transfer, the poor to fair embryos were shown to result in approximately half of the pregnancies achieved by the excellent to good embryos.[15] An unfertilized ovum (UFO) is the classification if there is no sign of cleavage (1 cell) or 2 - 6 fragments are without nuclei in single-ovulating females (quite rare). Embryos having blastomeres with nuclei but too underdeveloped or retarded to be considered viable embryos under the scheme of Lindner and Wright were designated degenerate (Deg) and would not have been expected to result in a pregnancy. Following classification for fertilization status/embryo quality, each embryo/ovum was examined at 1000x magnification for number and quality (morphology) of accessory spermatozoa. This required partial digestion and spreading of the zona pellucida on a microscope slide.[5]

The Nature of Accessory Sperm in Single-Ovulating Cows and Attempts to Alter their Numbers

Figure 5 shows the distribution of accessory sperm in embryos/ova of artificially inseminated single-ovulating cows. These data come from a series of experiments utilizing semen of variable quality, but quality within a range acceptable for use in AI.[5] As may be noted, the distribution in numbers of accessory sperm per embryo/ovum is highly skewed, having an average of 11.2 sperm per embryo/ovum, a median (50 percentile) of 2.0 sperm per embryo/ovum, and a mode (most common value) of 0 sperm per embryo/ovum. Of reproductive importance is the association of accessory sperm number with the fertilization status and embryo quality, presented in Table 1. Not surprising was the lack of accessory sperm in unfertilized ova, reported earlier.[32] However, the positive relationship of accessory sperm numbers (best reflected in median values) with embryo quality is significant and of importance reproductively since degenerate embryos would not be expected to result in pregnancy and, as stated earlier, fair to poor embryos would not provide for pregnancies at the same level as excellent to good embryos.[15] Also important in the interpretation of accessory sperm data is the high and heterogeneous variability from cow to cow associated with the average accessory sperm number (expressed as standard deviation in Table 1). As may be noted, the standard deviation appears to be positively associated with the mean. On the basis of these data, a series of attempts (experiments) were undertaken to increase accessory sperm number (median values); but also recognizing that a positive impact on fertility to artificial breeding could be achieved by reducing the cow to cow variability or increasing the mode in accessory sperm per embryo/ovum (Table 2).

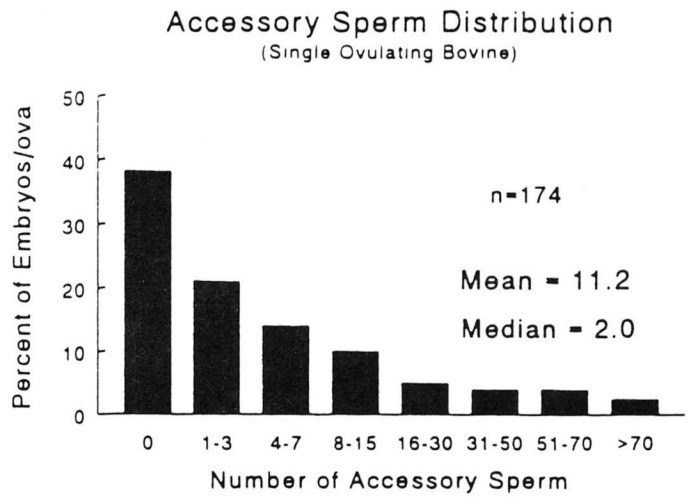

Figure 5. Frequency distribution of accessory sperm per embryo/ovum in single-ovulating cows.[5] In this type of highly skewed distribution, the median value (50 percentile) becomes more important than the mean and is therefore used to judge treatments or conditions affecting accessory sperm.

Table 1. Relationship of accessory sperm per embryo / ovum to fertilization status and embryo quality.[5]

Fertilization status/ Embryo quality	n	Mean ± SD	Median
Excellent/good	69	17 ± 29	5.0
Fair/poor	42	16 ± 29	3.5
Degenerate	21	5 ± 9	1.0
Unfertilized	42	0.3 ± 1	0

Median values are different ($P < .05$).

Table 2. Efforts to raise accessory sperm number per embryo / ovum.

Effort	Outcome	Ref
• Block retrograde sperm loss	No effect	(5)
• Frozen vs Fresh semen	No effect	(21)
• Extender comp (Milk vs EY)	No effect	(Unpub)
• Sperm microencapsulation	Negative	(19)
• Select male	Positive	(21)
• Semen dosage	Positive	(5, 21)
• Seminal plasma	?	

Blockage of retrograde loss of sperm at insemination had no effect on any accessory sperm value.[5] This effort was prompted by the work of Mitchell *et. al.*[18] They

showed that nearly 90% of the recoverable spermatozoa following an intrauterine insemination was in the expelled or vaginal mucus within 6 hours of insemination. In our study,[5] insemination was carried out through a catheter equipped with an inflatable cuff which was inflated in the cervix just prior to insemination and was permitted to remain in place in one experiment for 1 hour and in a second experiment for 3 hours post insemination. Conventional inseminations served as controls. Despite the fact that our method of reducing retrograde loss of sperm was ineffective, the fact remains that such sperm loss in AI (90%) is quite significant. In view of the low accessory sperm numbers associated with unfertilized ova and low quality embryos in cattle, it is important that other methods of reducing retrograde sperm loss be sought.

Surprisingly, the use of fresh semen gave no advantage over frozen semen in any accessory sperm value.[21] Earlier research in other laboratories, comparing frozen and fresh sperm with respect to sperm transport to and retention in the female oviduct, favored fresh semen.[16,17,25] We thought that this difference might be reflected in accessory sperm numbers. The fact that it wasn't, suggested that our cryopreservation methods today had been significantly improved over those used at the time the above cited work was carried out (prior to 1978). Since this time (1978), the development of the straw, along with nitrogen vapor freezing, liquid nitrogen storage and rapid thawing have resulted in a cryopreserved product more closely resembling fresh semen.

Extender composition, particularly milk vs egg yolk-buffered extenders, has historically received research attention regarding sperm survivability and fertility derived from their use. Arguments supporting the use of each can be found readily. Relatively recent research in our laboratory attempting to determine the method by which sperm naturally deposited in the vagina enter the uterus revealed that they arrived against the uterine wall after following relatively deep privileged paths within continuous cervical grooves originating in the fornix vagina.[20] We thought that an emulsion such as milk, particularly with sufficient butterfat, might make the inseminate have more affinity for the uterine wall ("Pepto-Bismol effect") than for the cervical mucus (located in the cervical and uterine lumen) which seems to destine the sperm for retrograde removal. In this study, semen was frozen in egg yolk citrate - glycerol, but mixed post thaw (just before insemination) with milk - glycerol extender having fat levels sufficient to make the inseminate either 3.5% or 8.3% fat. Neither fat level affected accessory sperm numbers when compared with the egg yolk citrate - glycerol control (unpublished).

A heterospermic study involving protamine sulfate microencapsulated vs unencapsulated spermatozoa resulted in a negative effect of encapsulation on accessory sperm numbers.[19] However, this study, conducted using PGF2a synchronized cows, was the first effort showing that encapsulated sperm were transported in the female and were capable of becoming accessory sperm and presumably, fertilizing sperm. Preliminary homospermic studies (where encapsulated and unencapsulated sperm are inseminated separately) indicate that encapsulated sperm may be equal to or better than unencapsulated sperm in fertility (non return) of CIDR-B synchronized cows.[22] More research is necessary in altering the capsular materials as well as evaluation of encapsulated semen inseminates with respect to time of breeding in relation to ovulation. Only then will we best understand the advantage of this new microencapsulation technology in artificial insemination.

The effect of semen dosage on accessory sperm number is presented in Table 3. These experiments were conducted in two separate trials. In the first trial, increasing dosage from 20 million to 40 million sperm per inseminate was without effect on accessory sperm number.[5] In this experiment, semen of lower quality was intentionally used with the idea that differences due to dosage would be more evident because a lower variation in accessory sperm number among cows could be expected. In the second trial, 20 million sperm was compared with 100 million sperm per inseminate.[21] The high dose resulted in a 9 fold increase in accessory sperm number (median value of 3 vs 27) and improved percentage of embryos/ova with accessory sperm (Table 3). The fertilization status/embryo quality was also significantly improved by the high dose (Figure 6) as was hypothesized from earlier work which showed a positive relationship between accessory sperm number and both fertilization status and embryo quality.[5] A legitimate question we should raise here is: Why would such a marked effect of semen dosage on fertility (as observed in this study) not have been recognized in the early developmental period of artificial insemination? The effect of semen dosage on fertility to artificial insemination using fresh or frozen semen was thoroughly reviewed in recent years (6 and 24, respectively). In both cases, sperm dosages studied ranged from below 1 million to 40 million cells per inseminate. For both frozen and fresh semen, fertility maximized between 10 and 30 million sperm per dose (dependent upon the bull, semen quality, and preservation system) suggesting that higher levels would be to no avail. In a personal communication with one of these reviewers (R.H. Foote, Cornell University) concerning this puzzlement, he recalled the early work involving semen dosages of 100 million cells and higher (but not able to cite them), as generally resulting in depressed fertility when con-

Table 3. Effect of sperm dose on accessory sperm.

Sperm dose		Accessory sperm per embryo/ovum		Emb/ova with acc sperm	Fert
($\times 10^6$)	n	Median	Mean ±SD	%	%
20	42	0	8 ± 22	52	66
40	39	1	10 ± 17	59	69
20	38	3	29 ± 63	79	79
100	38	27	38 ± 38	92	97

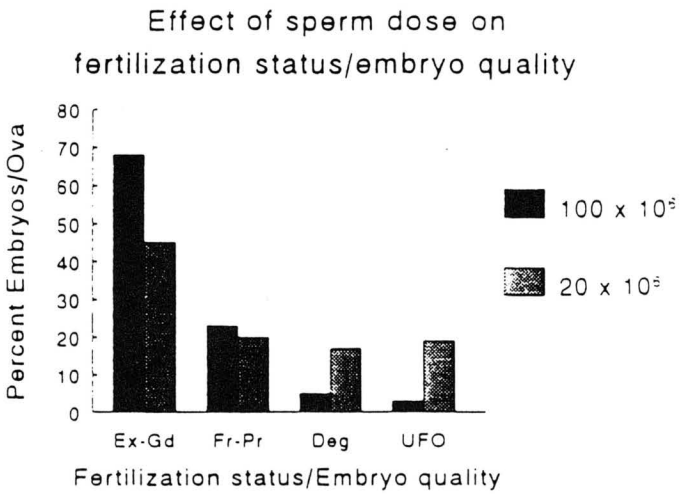

Figure 6. Effect of insemination with 100 x 10^6 sperm and 20 x 10^6 sperm on fertilization status/embryo quality in single- ovulating cows. The shift in viable embryos (classified excellent to good and fair to poor) to degenerate and unfertilized due to the lower dose was significant ($P < .05$). n = 38 each for the high and low dose.[21]

trasted with lower dosages. However, he pointed out that the work at these high dosages was carried out before antibiotics were added to semen. It is well accepted that success of artificial insemination by intrauterine deposition received its greatest boost from the addition of antibiotics to semen, most bulls having sufficient pathogens in their semen to preclude a successful insemination past the cervix. Thus, we believe that reassessment of the dosage dogma by others would seem appropriate at this time. However, a word of caution is in order since semen quality can be easily compromised by processing (freezing) high dosages (sperm concentrations) unless certain precautions are taken. Major problems faced in processing semen at high concentrations are the potentials for detrimental rapid pH change prefreeze due to high sugar content of seminal plasma[1] and providing insufficient extender cryoprotection. Thus, attempts to take advantage of a high dosage effect should include close scrutiny of the resulting post-thaw or pre-insemination semen quality. This is particularly of importance to those in the ET industry where semen could be custom prepared for use on superovulated cattle.

The bull has a marked effect on the numbers of accessory sperm.[21] Differences among bulls at two semen dosages can be seen in Table 4. As may be noted, bull A achieves high levels of accessory sperm at both high and lower dosages, while bulls B, C, and D vary in accessory sperm numbers and differ markedly between dosages. These accessory sperm data could not be explained by the relatively small differences among these bulls in semen viability. On this basis, it was postulated that seminal plasma differences among bulls may account for differences in accessibility of sperm to the egg following insemination.[21] Seminal plasma was also considered to be important in view of the dosage studies cited above since improvement in accessory sperm did not follow a simple linear increase in sperm numbers per dose. Rather, it appeared possible that levels of seminal plasma equivalent to that accompanying sperm numbers between 40 and 100 million per dose could provide an important advantage regarding sperm accessibility to the egg. A small study, simply adding seminal plasma (50:50) to a conventional inseminate (20 million sperm) was undertaken to evaluate this possibility. The results, not completely conclusive, are presented in Table 5. Although the median accessory sperm number was increased by the seminal plasma, the high variation from cow to cow renders this apparent difference insignificant. It certainly does not represent the 9 fold effect we might have expected from the earlier study of semen dose, but it does seem to point in the same direction. More definitive studies regarding seminal plasma are underway using cauda epididymal sperm with and without seminal plasma. Cauda sperm have never been exposed to seminal plasma (from accessory sex glands) and therefore should give us the correct perspective regarding the importance of this chemically complex fluid to sperm transport or accessibility to the egg in the inseminated cow. Another point of interest regarding the potential involvement of seminal plasma in bull fertility was that shown by Killian[9,10] where specific seminal plasma glycoproteins were associated with bulls having high fertility and other glycoproteins were more common in bulls having low fertility.

Table 4. Interaction of sperm dosage and bull on accessory sperm number per embryo / ovum.

		100×10^6			20×10^6	
Bull	n	Median	Mean±SD	n	Median	Mean±SD
A	13	45	49±40	12	34	57±84
B	24	10	18±22	13	3	11±25
C	6	32	52±56	10	2	26±70
D	7	6	24±32	13	0	4±8

Table 5. Effect of adding seminal plasma (50:50) to inseminate on accessory sperm per embryo / ovum.

Semen	n	Median	Mean	Embryos/ova with AS (%)	Fert (%)
Control	32	2.5	19±6	75	81
Sem Pl	32	6.5	23±12	84	88

Seminal plasma (Sem Pl) added just prior to insemination.
All values are non-significant.

Accessory Sperm and the Superovulated Cow

A comparison of accessory sperm in single and superovulating cows is presented in Table 6. These data indicate that ova/embryos with accessory sperm and numbers of accessory sperm per embryo are greater in single than superovulated cattle. Also, numbers of accessory sperm per cow favors single-ovulating cattle indicating that sperm transport and retention is better in single-ovulating cows.[27] However, recent unpublished observations indicate that accessory sperm in superovulated ova may not be as efficiently retained for 6 days (when ova are non-surgically flushed from the uterus) as those in single-ovulating ova. In contrast to single ovulating ova, tracks indicating where accessory sperm may have entered are often evident in the 6 day-old superovulated egg. No such evidence of accessory sperm loss has been observed in single-ovulated ova at 6 days. In addition, Pronase digestion of the zona pellucida of superovulated ova requires only half the time that single ovulated ova require suggesting a difference in the nature of the zona. A comparison of Pronase digestion times and the standard deviations in time from cow to cow for single ovulated ova, superovulated ova, and ova recovered by follicular aspiration, followed by *in vitro* maturation (IVM ova) are presented in Figure 7. It is clear that zona "hardness" may be quite dependent upon the source of the ovum with single-ovulated ova being the hardest, IVM ova the softest and superovulated ova intermediate. There is also quite a high variation in Pronase digestion times from cow to cow within ova types. This variation in the nature of the zona pellucida across ova types as well as within types, from cow to cow, may be quite relevant to the now- recognized importance of the zona in the selection of competent sperm. [8]

Despite the apparent loss of accessory sperm by

Table 6. Accessory sperm: super vs single ovulation.

Characteristics	Superovulated	Single-ovulated
Cows (n)	24	44
Ova/embryos (n)	155	31
% Fertilized	65	84
% Fertilized ova with accessory sperm	10	61
Accessory sperm per fertilized ovum	1±2	21±30
Mean accessory sperm per cow	0.7	9.0

From (27).

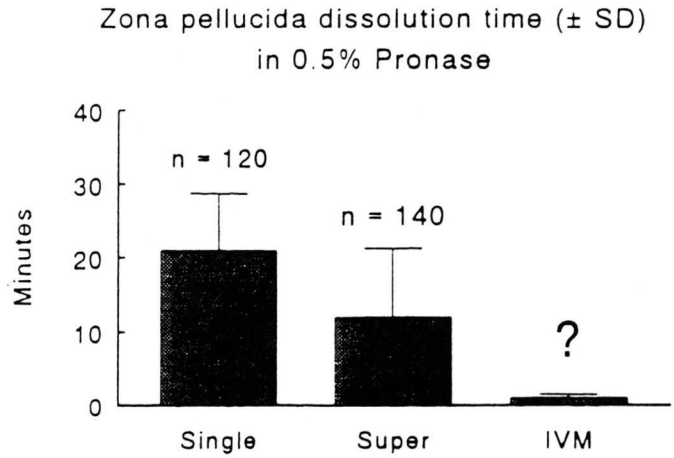

Figure 7. Mean time (± SD) required for the zona pellucida to visibly soften when exposed to a 0.5% Pronase in phosphate buffered saline. Note differences in the time for ova recovered from single-ovulating (single) vs superovulating cows (super) and IVM ova (ova recovered from ovaries by mechanical aspiration followed by in vitro maturation prior to IVF. These data were collected over a number of experiments while recovering accessory sperm from ova. The number of IVM ova was not recorded because they digest immediately upon contact with the Pronase.

the zona of the superovulated cow, there is still evidence that sperm transport may be limiting in the superovulated cow. Hawk *et al.*[7] showed that both ova with accessory sperm and fertility could be improved by raising the semen dosage (see Figure 8). Although the numbers of sperm inseminated were very high, the point is clear from their data that superovulated ova, like single-ovulated ova, are generally quite fertile and probably unfertilized because of insufficient contact with sperm.

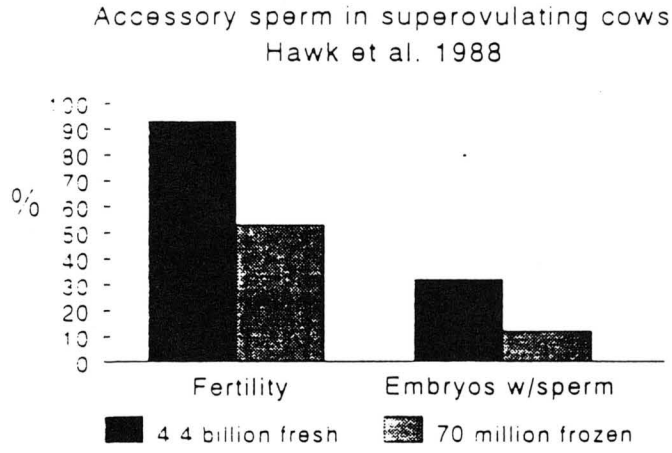

Figure 8. Effect of semen dosage on fertility and percentage ova with accessory sperm in superovulated cows.[7]

Uncompensable Semen Traits and Accessory Sperm

While the compensable aspects of semen quality address the ability of inseminated sperm to access the ovum (measured by numbers of accessory sperm per embryo/ovum), the uncompensable aspects address the quality or competence of the fertilizing sperm to continue or sustain the fertilization process or resulting embryo. This entails assessment of embryo quality along with that of potential fertilizing sperm (accessory sperm). We first addressed this problem by determining the quality of sperm constituting the accessory sperm population compared to that inseminated. Presumably, an accessory sperm could have fertilized the ovum if it was not out-competed by the fertilizing sperm. This work has shown that only sperm with normal shaped heads or subtle deviations in head morphology can constitute the accessory sperm population.[5,27] In addition, sperm with nuclear vacuoles/craters or pouches, including the diadem defect, if on otherwise normally shaped heads, gain access to the ovum at the same frequency as normal sperm in the same inseminate.[27] Data revealing the difference in sperm morphology of an inseminate compared to the resulting accessory sperm population is presented in Table 7. Semen from AI bulls containing such sperm (below average quality) were compared with average semen from the same AI organization (Table 8) and fertilization status/embryo quality was judged 6 days post insemination (Figure 9). Median accessory sperm number per embryo/ovum was essentially the same for both semen samples (2 for the average semen and 3 for the below average semen). It is clear from Figure 9 that the below average quality semen resulted in less fertilized ova and lower quality embryos than did the average semen. These data were obtained from single-ovulating cattle. It is not clear from this study which abnormalities were responsible for fertilization failure vs loss of embryo quality; however, such semen did have an uncompensable component.

More recent research in our laboratory involving the effect of thermal stress on abnormal semen content of bulls,[30,31] reveals that semen with specific abnormali-

Table 7. Percentage abnormal sperm: inseminate vs accessory.

Characteristics	Inseminate	Accessory
Normal sperm	26	53
Cratered (normal shape)	8	11
Tapered	16	3
Pyriform	2	0
Long	14	8
Asymmetrical	9	0
Sl Asymmetrical	25	20

(Sl) Slightly asymmetrical

Table 8. Pooled semen characteristics of average and below average experimental bulls.

	Semen Quality	
Semen Characteristic	Avg	Below Avg
%[5] Motile	40	30
% Intact Acrosomes	89	69
% Morphology		
Normal	81	61
Abnorm shaped head	8	17
Nuclear vacuole	5	10
Droplets	4	8
Abnorm tail	2	4

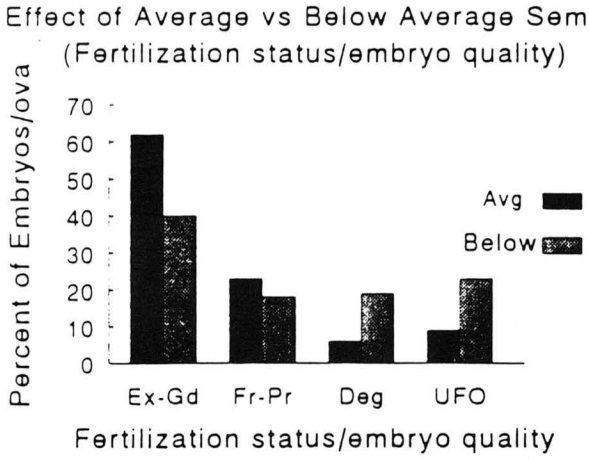

Figure 9. Effect of average (Avg) and below average (Below) semen on fertilization status/embryo quality.[5] The shift in viable embryos (classified excellent to good and fair to poor) to degenerate and unfertilized caused by use of below average semen was significant (P = .06).

ties can be produced following a mild 48-scrotal insulation. The type and chronology of the abnormalities in bulls collected at 3-day intervals following the scrotal insulation are presented in Figure 10. Since sperm with both random nuclear vacuoles and the diadem defect were found to readily access the ovum *in vivo*, we chose to compare the semen of a bull (A) that gave such semen following scrotal insulation (day + 21) with that he produced before scrotal insulation (day -3) in Trial 1. In Trial 2, we compared the semen of the same bull after scrotal insulation (day +9), but before appearance of spermatozoal abnormalities, with that he produced before scrotal insulation (day -6). The quality of the semen in both trials is presented in Table 9. The effect of this semen on the quality of embryos in superovulated cows may be seen in Table 10. Unfertilized ova have been omitted in order to more readily see the effect of the semen on embryo quality *per se*. As may be noted, the vacuolated - diadem semen of day +21 resulted in a significant increase in degenerate and fair-poor embryos at the expense of the excellent to good embryos when compared with the control semen (day -3). For the day +9 semen, there was no apparent shift in embryo quality when compared with the pre-insulation control (day -6). Thus, random nuclear vacuoles and diadem defects would appear to lower pregnancy rate by constituting an uncompensable factor in semen.[28]

Utilizing another bull from the same scrotal insulation study (Figure 10), semen containing protoplasmic droplets (day +9) was compared with that having decapitated sperm (day + 12) and pre-insulation control semen (day -6). The results are presented in Table 11. In this case the unfertilized ova are included since the major differences appear to be at that level with fertilization rates being greatly impaired by both post insulation samples. If this is the sole effect of this type semen, theoretically, raising the inseminate dose should overcome the problem (compensable trait). However, with superovulated animals there is often a bull effect in the category of degenerate/unfertilized ova (Deg/UFO) which is the category we use when we cannot say with certainty if the egg was or was not fertilized. The semen with protoplasmic droplets resulted in a distinct increase in this category. If this is a form of very early embryonic death, it would also constitute an uncompensable semen trait. Current studies are underway to clarify the true nature of these superovulated ova (Deg/UFO) with respect to fertilization status.

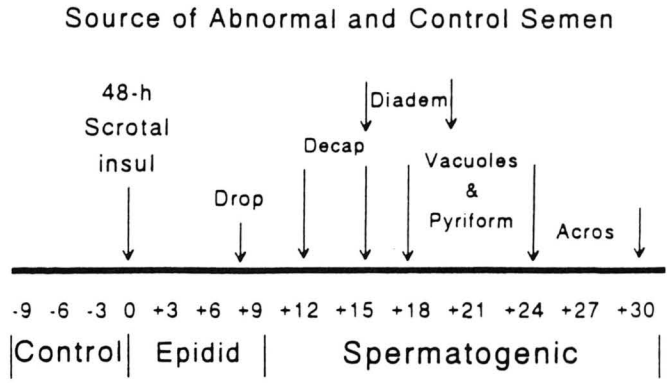

Figure 10. Schematic showing the approximate time of appearance of specific types of abnormal spermatozoa following a mild thermal insult of the testes by a 48-hour scrotal insulation.[30] From this study, both control and abnormal samples of the same bull have been taken to evaluate the uncompensable traits of semen.

Table 9. Experimental semen from a bull (A) responding to scrotal insulation with vacuolated and diadem sperm.

	Semen Quality			
	Trial 1		Trial 2	
Semen Characteristic	d -3	d+21	d -6	day +9
% Motility	50	50	55	40
% Normal	89	37	95	82
Random vacuoles	0	38	0	0
Diadem defect	0	19	0	0
Other abnorm	11	6	5	18

d -3 and d -6, semen from 3 and 6 days before scrotal insulation
d +21 abnormal semen
d +9 after scrotal insulation, but before appearance of abnormal semen.

Table 10. Effect of semen with sperm having nuclear vacuoles and diadem defects (Table 9) on embryo quality.

			Embryo Quality (%)			
Cows	Embryos	Semen	Ex-Gd	Fr-Pr	Deg	Deg/ UFO
7	90	d -3	74.4	11.1	14.4	0
8	85	d +21	38.3	21.2	35.3	4.7
7	87	d -6	66.7	11.5	17.2	4.6
9	141	d +9	56.0	22.5	19.9	2.1

Day +21 semen shows a significant shift in embryo quality ($P < .05$). All other apparent shifts are non-significant.

Table 11. Effect of proximal droplets and decapitated sperm on fertilization status / embryo quality.

			Fertilization status/ Embryo quality (%)				
Cows	Emb /ova	Semen	Ex/ Gd	Fr/Pr	Deg	Deg/ UFO	UFO
9	135	Cont	51.1	11.9	7.4	10.3	19.3
9	144	Drop	6.9	3.4	7.4	27.1	55.0
9	155	Decaps	12.9	9.7	9.0	14.9	53.5

Semen with droplets (drop) and decapitated sperm (decaps) are different in fertilization rate (%UFO) from the control (cont) ($P < .05$). Also, Deg/UFO category for the droplet semen is significant ($P < .10$).

Interaction of Uncompensable Factors with Sperm Numbers Inseminated

Finally, a word should be said about the positive relationship of accessory sperm number (median value) and quality of embryo (Table 1). This is apparently a real phenomenon since sperm dosages resulting in increased numbers of accessory sperm also improve embryo quality along with fertilization status.[21] Although we can only speculate at this point, in vitro work from laboratories dealing with other species sheds some light on why we may be observing this in artificially inseminated cattle.[8,11,13] These studies have shown that spermatozoa with abnormal heads can penetrate the vestments of the ovum, but do so less efficiently than normal sperm. The recent work of Howard *et al.*[8] in felids is the most compelling evidence for this phenomenon. They have shown that abnormal sperm can bind to the zona, however, penetration through the zona results in a reduction of abnormal forms with those reaching the innermost zona layer and the perivitelline space, being basically normal. Since accessory sperm are thought to represent the number of available fertilizing sperm, this number would also reflect the degree of competition among potential fertilizing sperm. In this regard, semen having uncompensable traits (abnormally shaped sperm) would perform differently depending upon the degree of competition at the ovum when fertilization takes place, thus the observed positive association of accessory sperm number and embryo quality. It would also make sense that impairment of the zona selection process against abnormal sperm (by being more permissive), as may be evident by softer zonas in superovulated and IVM ova (compared to single-ovulated ova), would make such ova more susceptible to embryonic mortality. An assumption in this entire concept would be that misshapen sperm are less competent than normally shaped sperm in the same sample. Although this seems to be the case, further research is necessary to confirm this important point.

Summary

What have we learned from accessory sperm and the ova/embryos from which they come?

1. The distribution of accessory sperm among ova/embryos of artificially inseminated cows recovered non-surgically 6 days post insemination is highly skewed with a relatively low median value (1-5 sperm per ovum/embryo) a mode (most common value) of 0, a mean of 11 and a range of 0 to 141.

2. The median number of accessory sperm per ovum/embryo is positively related to the fertilization status and embryo quality in single-ovulating cattle.

3. Many efforts to raise accessory sperm number have failed. However, very high doses of semen and the use of certain males have had a positive effect on this parameter with the expected outcome of both higher fertilization rates and improved embryo quality in single ovulating cows.

4. Morphologically, sperm gaining access to the ovum, *in vivo* as accessory sperm appear limited to those that are normal in shape or those with only subtle distortions of the sperm head. Those abnormal sperm having a full range in severity of nuclear vacuoles (craters) or diadem defects but otherwise normally shaped heads also gain full access to the ovum. Viable sperm in the same inseminates having more severe distortions of the head are not

observed as accessory sperm. Sperm with flagellar defects cannot be adequately addressed by accessory sperm morphology.

5. Use of semen with significant levels of viable sperm having abnormal shaped heads or nuclear vacuoles/diadem defects, results in decreased fertilization rates and increased proportions of low quality embryos in relation to controls (both single and superovulated cattle). This indicates that such semen contains uncompensable traits. In contrast, semen with high proportions of tailless sperm or sperm with protoplasmic droplets has the greatest impact by depressing fertilization rate, not embryo quality (presumably compensable).

6. In view of the assumption that numbers of accessory sperm reflect competition at fertilization, the positive relationship of accessory sperm number and embryo quality found in cattle suggests that abnormal sperm in an ejaculate may be incompetent after initiating fertilization. This concept is in harmony with findings in other species that abnormally shaped sperm can fertilize, but do so less efficiently than normal sperm.

7. Superovulated ova may be more vulnerable than single-ovulating ova to fertilization by abnormal sperm due to differences in the permissive nature of their zonas (based on digestion behavior and retention of accessory sperm).

From the standpoint of improving reproductive efficiency to artificial insemination (fertilization rate and embryo quality), accessory sperm data suggest that there is considerable room for improvement by finding methods or techniques to increase the median accessory sperm number per inseminate, and/or reduce the numbers of ova without accessory sperm or without evidence of sperm penetration (superovulated cows). Reducing variation in accessory sperm number among cows would also favor improved reproductive efficiency.

One should not expect a single semen test to predict the fertility of males/inseminates since there appears to be both compensable and uncompensable traits impacting reproductive efficiency. It appears that these two factors may differ among ejaculates/males/inseminates in any possible ratio or combination. It is also clear that the superovulated animal may be more sensitive to both deficiencies in that sperm transport as well as sperm selection may be impaired relative to the naturally ovulating female.

We should strive to recognize the uncompensable traits in semen and eliminate from use males chronically producing semen with such traits.

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FUTURE MEETINGS

American Association of Bovine Practitioners

1996	San Diego	September	12-15
1997	Montreal	September	18-21
1998	Spokane	September	24-27
1999	Nashville	September	23-26
2000	Rapid City	September	21-24