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New Approach to Estimating Bull Fertility

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NEW APPROACH TO ESTIMATING BULL FERTILITY

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Breeding soundness evaluations (BSE) are conducted to qualify bulls as potential satisfactory breeders. Guidelines for acceptable measures of scrotal circumference, sperm concentration, sperm motility, and the frequency of sperm with morphological abnormalities are compiled and published by the American Society for Theriogenology (Chenoweth et al., 1992). Unfortunately, according to statistics available from USDA, only 40% of eligible bulls are subjected to a BSE. In the real world, bulls with identical outcomes in terms of physical quality of semen will still vary in actual fertility whether used for natural mating or artificial insemination (A.I).

The beef industry has placed an emphasis on selection of bulls on the basis of scrotal size. Selection for that single trait will decrease the age of puberty in sons and daughters in subsequent generations. Scrotal size ultimately corresponds to sperms output, but it does not correlate highly with fertility per se. A good example is that we may have a large warehouse to store inventory. Without closer inspection, we may find that the inventory in that warehouse is mostly defective merchandise.

Methods have been developed to study features of sperm cells. As the percentage of abnormal cells in semen samples increase, fertility will decline. Some of those specific defects are highly heritable, so a general recommendation is to use 25% total abnormal cells as the upper limit.

The region on a sperm cell located at the tip of the head is referred to as the acrosome. It contains enzymes which are used by the sperm to undergo the final stages of fertilization. Freshly ejaculated sperm cannot fertilize. For most mammals, sperm must reside in the female reproductive tract for about 8 hours to undergo a process termed "capacitation". The final step of capacitation involves erosion of the acrosome, releasing its enzyme contents. The process is called the acrosome reaction. A.I. companies routinely assess the status of the acrosome on sperm in thawed semen samples after a 3 hour incubation. It has been well established that bulls with higher fertility produce sperm where most of the cells have intact acrosomes after those incubations. The correlation between those measures is the trait most highly related to fertility of A.I. sires (Saacke and White, 1972). In theory, sperm with intact acrosomes under those conditions will not have enzymes leaching out prematurely, so capacitation can be completed in the vicinity of the ovulated egg.

In the early 1980's, our studies began by studying the functional importance of being able to initiate the acrosome reaction so a system of in vitro fertilization could be developed using bovine sperm and eggs (Lenz et al., 1983). We had to mimic ovulation steps for the eggs, and capacitation had to be coordinated for the sperm. Since sperm travel through the female reproductive tract in mucus, we had to conduct biochemical tests with mucus samples. Large carbohydrates in mucus would effectively cause capacitation. Those materials were chemically similar to heparin which is commercially available as an anticoagulant pharmaceutical. We soon discovered that if concentrations of those heparin-like compounds and sperm were held constant in a test-tube, the frequency of sperm that initiated the acrosome reaction correlated highly with fertility of A. I. sires (Ax et al., 1985; Ax and Lenz, 1987).

Heparin can be chemically modified to become radioactive. In that case, sperm from higher fertility males bind more radiolabeled heparin than lower fertility counterparts. This is true for mammals other than cattle, including humans (Marks and Ax, 1985; Miller et al., 1988).

Since 1990, we have developed a method to be able to identify what components on sperm bind heparin to induce the acrosome reaction. Several families of proteins have been isolated, purified, chemically cut into fragments, and studied in detail.

Mice were injected with those proteins to cause an immune response analogous to us receiving a vaccination. The mouse that made the greatest amount of antibodies was sacrificed, and individual spleen cells were harvested and fused to cancer cells. From those procedures, 38 different cell lines were developed which can be stored frozen in liquid nitrogen. Each cell line produces a unique antibody which can be evaluated for diagnostic potential.

One of the proteins which binds to sperm during ejaculation has been named fertility-associated antigen (FAA). Table 1 illustrates the difference in fertility between bulls that produce sperm with or without detectable FAA on their sperm surfaces. Over a 6-year span of breeding trials at King Ranch, FAA-positive bulls were 19% higher fertility than their herdmates lacking FAA. In all of those field trials, bulls were used for natural matings for a 60 day breeding season at a ratio of 1 bull per 25 cows. There were approximately 12 bulls per pasture that all had been pre-screened for similar protein patterns on sperm.

Table 1: Fertility of cows bred to bulls with or without detectable FAA on sperm. From Bellin et al., (1994; 1996; 1998).

	FAA in Sperm Membranes		
	Present	Absent	Total Numbers
No. Bulls	242	192	434
No. Cows Bred	5,317	4,497	9,198
No. Cows Pregnant	4,497	2,572	7,069
Pregnant (%)	85	66	77

Table 2 summarized results from a study where bulls were evaluated for serving capacity (desire to mate), and sperm were tested for FAA presence. From the results below, the ideal situation would be for us to rigorously evaluate sperm proteins and serving capacity. Is it not surprising why we see such wide ranges in fertility in the field based upon the data in Table 2?

When DNA-fingerprinting is used to identify calves from multiple-sire pastures, it routinely turns out that a small proportion of the bulls sire the majority of calves. Therefore, every bull we assign to do breeding chores should have the potential to be a high fertility sire if we want to propagate his genes into the next generation with some degree of success.

Table 3 contains data which will be published soon (Sprott et al., 1999). With A. I., serving capacity does not enter into the equation. In that situation, FAA becomes the source for comparison, and its presence on sperm from A. I. beef sires translated into 16% higher fertility at first service (Table 3). We took the liberty to calculate what A. I. outcomes would be after 3 inseminations (60 days) if second and third service pregnancy rates were the same as first service for individual bulls. Clearly, results are almost identical (Table 3) to data in Table 1 where a 60 days breeding season was employed.

A logical question at this junction is whether FAA status of sperm is heritable. Since 1992, cows in the nucleus herd of Santa Gertrudis at King Ranch have only been bred to FAA-positive bulls. Their daughters that made it into the nucleus herd were also only bred to FAA-positive bulls. Between 1992 and 1998, 20% more calves were born in the first 20 days of the calving season from adopting that breeding strategy (Table 4).

Since FAA is a protein, and individual proteins are encoded by individual genes, tools of modern biology should enable detailed studies of the FAA gene to be undertaken. Those experiments are underway from several different dimensions at the time this manuscript was being written. FAA is composed of 296 amino acids. It is not decorated with sugar side-chains as many biologically active proteins are (McCauley et al., 1999). The coding portion of the FAA gene should be about 900 bases of DNA. To date, 643 of those DNA bases have been sequenced. Within that sequence, a mutation has been found in a sterile bull. From human prostate tissue, 4 mutations have been found, with 1 of those identical to the sterile bull. A computer simulation predicts that the mutated form of FAA would have a different structure than FAA normally does. That probably accounts for why FAA is not detected on sperm from bulls with lower fertility. We envision a time in the near future where DNA-based screening for fertility potential might center on FAA or some other fertility-related proteins (Cancel et al., 1997; Killian et al., 1993) currently being studied in other labs. Genes tend to be highly conserved. If the FAA gene structure corresponds to bull fertility, it could immediately be studied with heifers (cows) and ultimately in other farm animals and mammals. For infertile couples, semen fortified with an FAA-like additive may hold therapeutic value to produce a child before other medical interventions are attempted.

Table 2: Fertility of Santa Cruz bulls grouped according to FAA and serving capacity profiles during the breeding season. From Bellin et al., (1998).

Bull profiles		Cows pregnant, %						
		Breeding season, d[e]						
FAA[a]	Serving capacity[b]	No. of cows[c]	BCS[d]	1 to 20	21 to 40	41 to 60	Fertility, %	Differences in fertility[f]
Positive	High	270	4.2	50	19	18	87[x]	—
Negative	High	143	4.8	45	13	20	78[y]	9
Positive	Low	238	4.3	29	13	27	69[z]	19

[a] All bulls were 2 yr. Old Santa Cruz. Bulls were grouped according to presence of fertility-associated antigen (FAA); on sperm membranes and serving capacity.

[b] Serving capacity was determined by observing and counting the number of times a bull mated (defined as intromission and (or) apparent ejaculations) estrus-synchronized heifers during a 20-min period. Bulls that mated with two or more estrus-synchronized heifers were classified as high serving capacity; bulls that did not mount any heifers were classified as low serving capacity. Serving capacity tests were performed a few weeks before the breeding season.

[c] Bulls were bred to 3 yr. Old crossbred cows (½ Simmental, ¼ Hereford, ¼ Brahman). Cows were being bred to produce their third calf.

[d] Average body conditions scores (BCS) for cows were estimated.

[e] The day of pregnancy was estimated by approximating the age of the fetus when cows were checked for pregnancy.

[f] Differences in fertility were calculated by subtracting the fertility of each group from the fertility of the group with FAA present and high serving capacity.

[x,y] Values differed ($P < .05$).

[y,z] Values differed ($P < .01$).

Table 3: Number of females pregnant to first A.I. service and projected number pregnant after 3 services to semen from FAA negative (-) or FAA positive (+) sperm.

No. Females	No. Bulls	Bull FAA Status	No. Preg. to 1st service (%)	Proj. No. Preg. by 3 Serv. (%)
386	7	negative	192 (49.7)	283 (73.3)
764	18	positive	501 (65.6)	673 (88.1)
Total 1150	25			

Table 4: Distribution of calving season in the nucleus herd at King Ranch. Cows were bred to FAA positive bulls, and their retained daughters have only been bred to FAA positive bulls.

Days of Calving Season	Prior to FAA testing - 1991 223 head		1995 262 head		1998 489 head	
	%	Avg. Wean Wt.	%	Avg. Wean Wt.	%	Avg. Wean Wt.
1-20	43.0	590	51.5	586	61.2	569
21-40	35.4	542	31.3	539	25.3	535
41-60	16.1	476	10.3	471	11.1	490
	94.5		93.1		97.6	

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