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Insemination factors related to timed AI in cattle

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Abstract

Six-day-old bovine ova/embryos were recovered non-surgically and used as biomonitors to evaluate time of artificial insemination. These embryos/ova provided information regarding fertilization status and embryo quality, as well as quantitative and qualitative data regarding associated accessory sperm. Both sperm access to the ovum (addressed by accessory sperm) and fertilization status/embryo quality were important in addressing pregnancy rate for specific intervals from the onset of estrus to insemination. Based on these biomonitors, early insemination failed to achieve optimum pregnancy rate due to inadequate access of sperm to the ovum (i.e., low fertilization rate, manifested by low accessory sperm numbers). However, embryo quality was high in early inseminations, which favors pregnancy. Late insemination failed to achieve optimum pregnancy rate (due to reduced embryo quality), however, sperm access to the ovum was highest. Thus, the selection of an insemination time to achieve optimum pregnancy rate appeared to be a compromise between the two extreme intervals. For timed-AI programs, consideration of the time of ovulation (and its variability) becomes important, in addition to conventional considerations, such as semen handling, site of insemination, and bull selection.

Keywords: Time of insemination; Timed insemination; Artificial insemination; Accessory sperm; Embryo quality

1. Introduction

In addition to the requirements for healthy, well-managed cattle, and the appropriate administration of pharmaceuticals, many other factors affect the success of an AI program based on estrous synchronization or timed insemination. Considering the economic investment in semen and other inputs, success must be judged on the basis of pregnancy rate to the first AI. Also, a good first service pregnancy rate response usually signifies conditions are good for second service. Additional key factors to be considered as impacting pregnancy rate to first service are semen quality (primarily dependent on choice of bulls), the timing of insemination, and the competence of the inseminators in semen handling and placement. In most breeding strategies, whether estrous synchronization is employed or not, semen quality, placement, and timing of AI 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 ovum competence) has yielded insights to the problems associated with attempts to optimize pregnancy rate to AI. The objective of this presentation is to provide some of these insights, particularly those associated with the semen/bull and the timing of AI.

2. Accessory sperm and their implications for pregnancy rate

A research area yielding valuable insight into semen and management-related problems involved accessory

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sperm; these are sperm trapped in the zona pellucida, one of the important ovum vestments which sperm must penetrate in order to fertilize. Although only one sperm fertilizes an ovum, there is a range in the number of sperm simultaneously competing for this honor. Once the fertilizing sperm enters the ovum 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, sperm competing for fertilization during that short interval provided by the ovulated, fertilizable ovum.

During several years of experimentation in our laboratory, we recovered over 1000 ova/embryos from single-ovulating cows 6 days after AI (nearly 30 bulls were used). The distribution of accessory sperm in the zona pellucida of these ova and embryos was very skewed, with an average, median and mode of 12.0, 2.4, and 0 sperms per ovum/embryo, respectively (Fig. 1). Therefore, clearly only a few sperm competed for fertilization at a given time. Of reproductive interest, was the association of accessory sperm number per ovum/embryo to fertilization status and embryo quality; it was best described by the median number (50th percentile) of accessory sperm per ovum/embryo (Table 1). Clearly, unfertilized ova were simply sperm hungry, having a median accessory sperm number of 0.

Furthermore, embryo quality was associated with median accessory sperm number; good to excellent embryos had a higher accessory sperm number than degenerate or fair to poor embryos, but the mode remained 0, regardless of embryo quality. Despite the rather small difference in median number of sperm, we inferred that larger numbers of accessory sperm were most likely associated with higher embryo quality (due to greater competition among potential fertilizing sperm at fertilization). There is evidence this competition favored more competent sperm (i.e., sperm selection may also occur at the zona pellucida [2], as well as at other locations in the female tract [1]). Therefore, 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 cows that were artificially inseminated (Fig. 2). We concluded that nearly 10 sperms/embryo were necessary to reach the maximum embryo quality index; thereafter, increasing accessory sperm numbers had no further influence on embryo quality. Regardless of embryo quality, it was noteworthy that the mode in accessory sperm number remained 0, i.e., the most common occurrence was one sperm per ovum, the fertilizing

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

Fertilization status/ embryo quality	No.	Mean ± S.D.	Median
Excellent/good	449	24.5 ± 44.1	7
Fair/poor	213	17.2 ± 32.2	5
Degenerate	80	13.5 ± 38.1	1
Degenerate/UFO	12	2.7 ± 5.7	0.5
Unfertilized	173	1.6 ± 16.5	0

Embryo quality was based on Lindner and Wright [3], as modified for degenerate embryos by DeJarnette et al. [4].

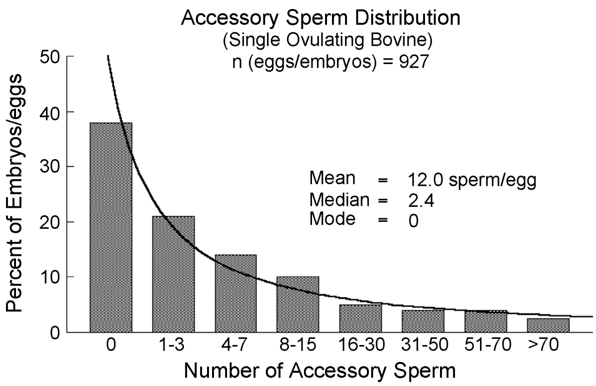

Fig. 1. Frequency distribution of accessory sperm per embryo or ovum in artificially inseminated single-ovulating cows. Quality and quantity of semen used varied, but was within acceptable standards for commercial artificial insemination. Distributions were similar for individual experiments utilizing both frozen and fresh semen [1].

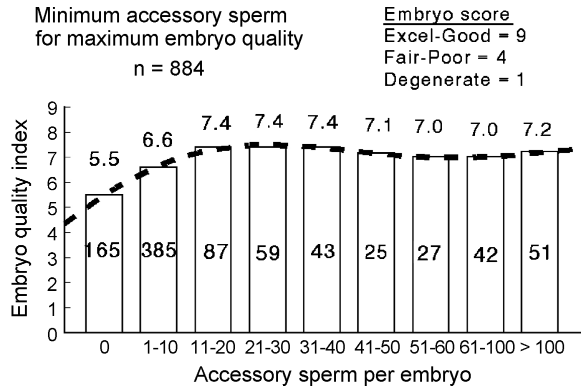

Fig. 2. Numbers of accessory sperm required to maximize embryo quality index for 6-day-old embryos (morulae) derived from AI of single-ovulating cows. Embryo grading was according to [3], as modified by DeJarnette et al. [4]. Embryo quality index was the average embryo quality based on the numerical score listed above. A minimum of approximately 10 accessory sperm/embryo was required to maximize embryo quality index. The number within each bar is the number of embryos recovered in that accessory sperm category.

sperm, again suggesting that very few sperms approach the ovum at any point in time. Conversely, this exercise emphasized the importance of semen handling and placement to achieve threshold or above threshold number of sperms to the ovum (i.e., approach 10 sperms/ovum) necessary to maximize both fertilization rate and embryo quality for a general population of bulls. Furthermore, the large variation in accessory sperm (expressed as SD) within and across fertilization/embryo status categories (Table 1) precluded the use of accessory sperm numbers for predicting male fertility, particularly at numbers of observations (ova) practical using this approach. The large variation emphasized our knowledge deficits regarding 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. Furthermore, this concept has relevance for evaluating time of insemination.

It is important to appreciate how embryo quality (as judged) affected pregnancy rate, particularly in assessing uncompensable semen traits and reproductive strategies. The best data are those of Lindner and Wright [3], who developed the embryo scoring system used in the data presented above. They reported that embryos classified as excellent to good yielded twice as many pregnancies (after transfer to recipients) as those classified fair to poor. Presumably, much of this difference in embryo performance would be similar for embryos permitted to remain in utero. Of course degenerate embryos and unfertilized ova produce no pregnancies under any circumstance. Based upon the median number of 2.4 accessory sperms per ovum/embryo (Fig. 1) and the threshold requirement of nearly 10 sperms per ovum/embryo to optimize embryo quality (Fig. 2), efforts to raise accessory sperm number have been undertaken and previously reviewed [1,5]. These efforts will not be repeated here, except to emphasize two of the major positive factors impacting accessory sperm numbers per ovum/embryo relevant to estrous synchronization and timed insemination, namely, choice of bull and time of insemination.

3. Effects of bull and time of insemination on sperm access to the ovum and embryo quality

Even when cows are bred at approximately 6–16 h following the onset of estrus (in accordance with conventional recommendations), there was considerable variation among bulls with respect to numbers of sperm accessing the ovum [6]. Data from this study comparing four bulls is presented in Table 2. Clearly, Bull A had high ovum access, as denoted by the high accessory sperm number (median of 40 sperms per ovum) compared to the other three bulls. Assuming these four bulls were similar in uncompensable traits, it would be expected that bulls similar to Bull A would perform as well at low sperm dosages as at normal dosage, and/or this bull would be less vulnerable than other bulls to inseminator error in semen placement and handling. Such a bull would be considered to have little to no compensable deficiencies and would 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 pregnancy rate in these two bulls would be more vulnerable to dilution rates, inseminator competence, and timing of insemination. Based on a median of two sperm per ovum, 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. However, as discussed in my accompanying presentation, there are compensable differences among bulls that we still do not understand and can only determine by fertility data from AI of adequate numbers of cattle at known semen dosages.

Table 2
Accessory sperm differences per embryo/ovum among bulls (constant insemination dosage)

Bull	No.	Median	Mean ± S.D.
A	25	40	53 ± 61
B	37	8	15 ± 23
C	16	13	36 ± 65
D	20	2	11 ± 16

Derived from Nadir et al. [6].

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 in my accompanying paper. Abnormal sperm in the semen reflect the health of the spermatogenic process, and in particular, the health of the DNA contributed to the embryo by the male [4]. DeJarnette et al. examined 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 (Fig. 3). Clearly, below-average semen produced fewer

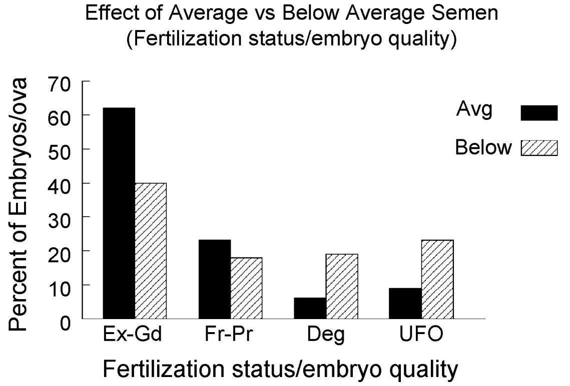

Fig. 3. Effect of average and below-average semen (based upon content of abnormal sperm) on fertilization status/embryo quality in single-ovulating cattle. Fertility and embryo quality were influenced by the semen, as noted in the shift in distribution across categories ($n = 21$ and 22 for the average and below-average semen, respectively) [4].

excellent to good embryos and greater numbers of degenerate embryos and unfertilized ova when compared to semen of average quality, again emphasizing the importance of uncompensable seminal traits to pregnancy rate. Today, bulls in AI centers 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 detect changes in a bull's spermatogenic status. Sperm morphology evaluation is also one of the main components of the breeding soundness evaluation of bulls destined for natural 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 [7] Again, abnormal sperm morphology is the best method of recognizing the consequences of abnormal testicular thermoregulation.

The effect of insemination time on numbers of accessory sperm, fertilization status and embryo quality was studied by Dalton et al. [8]. In this experiment, the HeatWatch® system was used to determine the time of AI for each cow. In this estrus detection system, an electronic device is placed on the rump of the cow and a signal transmitted when the device is activated for 2 s 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 h following the first mount, for estrous cycles with either spontaneous or prostaglandin-induced luteolysis [9]. In this study, AI was planned 0, 12 or 24 h after the onset of estrus (first mount). However, due to logistics associated with monitoring the computer every 3 h followed by retrieving the cow for breeding, the actual times of AI were: 2.0 ± 0.9, 12.1 ± 0.6, and 24.2 ± 0.7 h following the first mount, respectively. Six days after AI, the embryo was recovered non-surgically and examined for fertilization status/embryo quality and numbers of accessory sperm, as described [4]. Artificial insemination was to one of three bulls used at random and balanced in number of resulting ova/embryos recovered for each time of insemination.

Clearly, accessory sperm number per embryo/ovum was favored by breeding later, rather than earlier (Table 3). Fertilization rate and embryo quality are presented for each insemination interval (Fig. 4); as expected, increasing fertilization rate followed increasing accessory sperm number. Fertilization rate was favored by breeding late (24 h after the onset of estrus) and poorest by breeding early, near onset of estrus. However, embryo quality was high with inseminations at or near the onset of estrus, but decreased to low quality with insemination 24 h after the onset of estrus.

Therefore, we inferred that optimum reproductive efficiency (pregnancy rate) was a compromise using our current techniques and recommendations. Early insemination resulted in lower fertilization rates (but good embryo quality), whereas late insemination reduced embryo quality (but the fertilization rate was good). Thus, the intermediate time of approximately 12 h after the onset of estrus was optimal when using a precise method for determining estrus onset (Fig. 5). This optimum was verified in field studies using HeatWatch® [10]; 6–16 h after the onset of estrus provided the best pregnancy rates. The basis for pregnancy rate failure by breeding late (24 h post-onset) could be due to an aging ovum, if we assume ovulation occurs 27.6 ± 5.4 h post-estrus onset (as detected by HeatWatch®). Sustained sperm transport to the site of fertilization in the oviduct requires a minimum of 4–6 h following insemination in the cow [11]. Thus, sperm arrival in the oviduct following a 24-h insemination would be 28–30 h post-estrus onset, after many ova

Table 3
Effect of interval from onset of estrus to AI (25×10^6 sperm/dose) on accessory sperm per embryo/ovum ($n = 39$ cows/interval)

Interval (h)	Mean ± S.D.	Median	Fertilized (%)
0	9 ± 23	1	66
12	21 ± 46	2	74
24	33 ± 53	4	82

Ovulation occurred 27.6 ± 5.4 h after the onset of estrus. Derived from Dalton et al. [8].

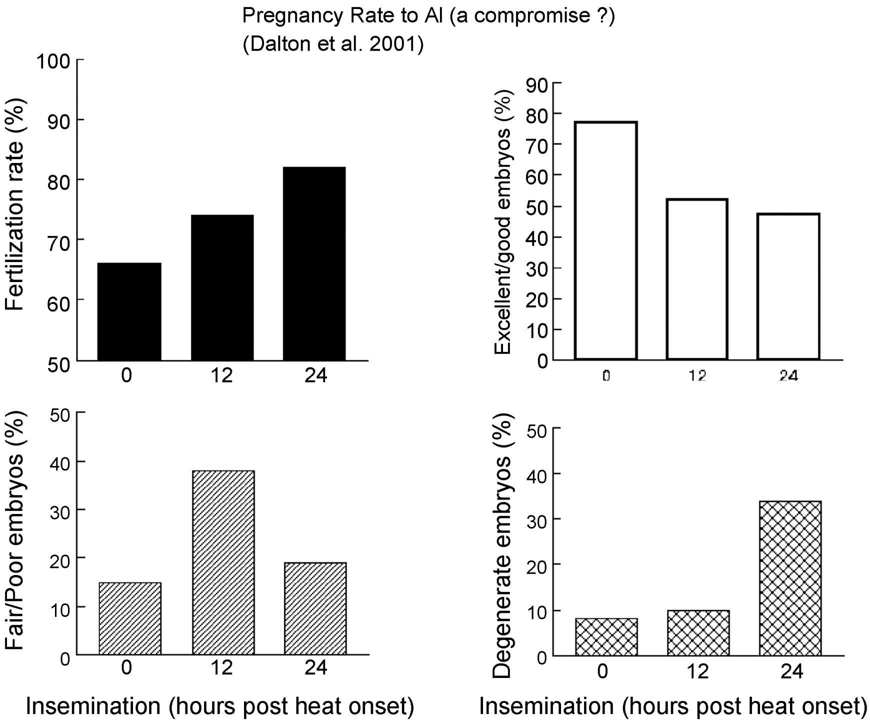

Fig. 4. Effect of time of AI following onset of standing estrus (HeatWatch®) on fertilization status and embryo quality judged 6 days after AI ($n = 117$). Modified from Dalton et al. [8].

were already ovulated. Therefore, in the current study, many ova awaited the arrival of sperm, thereby attributing degenerate embryos to late insemination (aging ovum) rather than a male-related uncompensable trait. Conversely, 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 (Fig. 5). The correct explanation is probably a combination of the two, with further study needed.

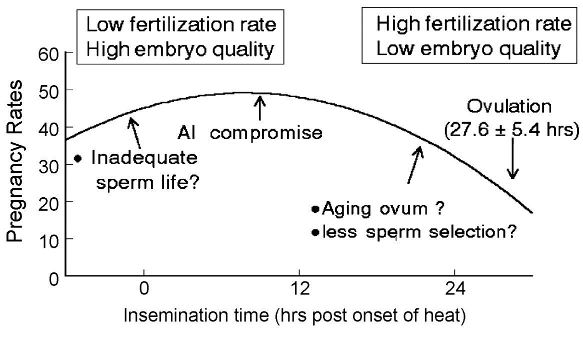

Fig. 5. Calculated pregnancy rate (from data in Fig. 4) and based upon the ability of embryos classified excellent to degenerate to constitute a pregnancy (according to Lindner and Wright [3]). Artificial insemination was a compromise, with early inseminations being inadequate due to high levels of unfertilized ova, and late inseminations characterized by poor embryo quality, most likely due to an aging ovum. However, high embryo quality appeared to be associated with early insemination and high fertilization rates were associated with late insemination [1].

4. Relevance of insemination time to new reproductive strategies and differences among bulls

Important to the insemination strategies employed with the burgeoning number of synchronization protocols is knowing the time of ovulation and its variability; this is vital to determine when to inseminate in relation to treatments or behavioral clues predicting ovulation. Based on the current data, AI must be late enough to maximize sperm access to the freshly ovulated ovum, but not so late as to ignore sperm transport time in the cow and risk the possibility of an aging ovum awaiting the arrival of sperm. Thus, in contrast to the study reported with ovulation at 27 h post-estrus onset, if a synchronization regime were to postpone ovulation until 30 or 35 h following estrus onset, the 24-h insemination could be the best in optimizing pregnancy rate (both fertilization rate and embryo quality). Clearly, controlling the CL ovarian

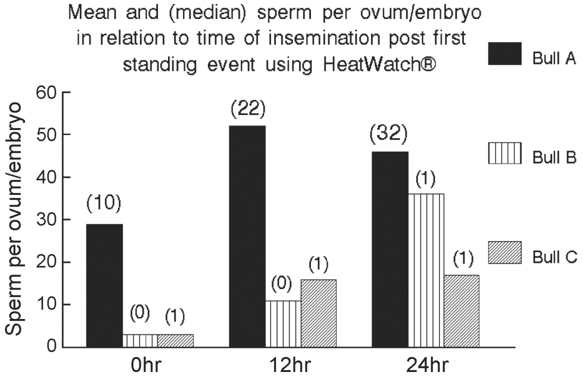

Fig. 6. Variation among bulls in sperm access to the ovum relative to time of insemination after the onset of estrus. Mean sperm per ovum/embryo is shown by the bars and the median number in brackets. Bull A had adequate numbers of sperm accessing the ovum at all breeding times, whereas Bulls B and C required insemination closer to ovulation [8].

follicular development, currently under intensive research, offers tremendous advantages in providing a highly synchronous and predictable ovulation.

Finally, I 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. [8] in Table 3 are shown (Fig. 6). 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 ovum. Unfortunately, and as you might expect, these data are difficult, time consuming, and expensive to acquire, and therefore are not readily available. The best approach is to have as much information as possible regarding bull differences, and knowing the expected time and variation in ovulation in order to choose an insemination time that maximizes fertility. Lastly, acquiring semen from a reputable source is the best protection against poor quality semen.

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