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THERIOGENOLOGY

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NORMAL DEVELOPMENT OF FETUSES RESULTING FROM HOLSTEIN SEMEN PROCESSED FOR SEX SEPARATION

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ABSTRACT

Semen from two high fertility Holstein bulls with extensive histories in artificial insemination was specially processed for the purpose of sexing and then frozen. The semen was used to inseminate 200 open crossbred beef heifers carefully selected for reproductive soundness from a much larger group. Animals were inseminated alternately with the semen from one of the two bulls and subsequently slaughtered. There were 96 fetuses, ranging in age from 63 to 88 days, which were carefully examined for general appearance, fetal crown-rump length and body weight. There was no effect of sire on fetal size, but the difference between fetal sexes was significant, with the male fetus being appreciably heavier. The regression of fetal length on fetal age was linear and the regression of fetal weight on fetal age was curvilinear. Fetal age accounted for 92% to 96% of the variation in fetal weight and also in crown-rump length.

Key words: Holstein, fetal development, sperm, sex separation

INTRODUCTION

There are several reports on gross fetal development in cattle (1-3). However, from a review of the literature by Evans and Sack (1) and examination of original reports, there were few fetuses at each of the various stages of gestation, and often fetuses were from heterogenous matings with unidentified sires. Beginning at about 60 days of gestation the fetus, *per se*, is a useful indicator of pregnancy, and the size of the fetus can be used to estimate its age as well as to predict the expected calving date of the dam (4,5). This is especially useful when the conception date is unknown because of pasture breeding or with multiple inseminations.

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In a study with "sex-separated" semen extreme precautions were taken to use sires of known background to provide semen for insemination on carefully recorded dates. The females, slaughtered under careful supervision 63 to 88 days after the last insemination, provided unusually well documented material for this study of gross fetal development.

MATERIALS AND METHODS

Two Holstein bulls provided by Eastern A.I. Coop., Inc. were selected because they had a history of high semen quality, excellent freezability of the spermatozoa and high fertility. They were used to inseminate 5488 cows in the routine operation of Eastern A.I. Coop., Inc. while this experimental study was in progress. Their 56-day nonreturn rate to first service was 74%. Semen for the present trial was processed at Eastern A.I. Coop., Inc. with equipment and procedures described previously (6). Spermatozoa selected to produce fetuses of only one sex were used in the experiment.

The females consisted of approximately 200 head of sexually mature open, physically sound and reproductively normal crossbred beef heifers, which were selected from a much larger group. They averaged 720 lbs. (327 kg.) upon arrival and all would have graded as high quality (M1, L1) cattle. These animals were moved to an isolated facility where they were doubly ear-tagged, well fed and carefully checked for estrus. Once normal estrous cycles were established they were synchronized with prostaglandin F_2 alpha^a and assigned alternately to be inseminated with frozen semen from one or the other of two bulls. If an animal returned for reinsemination, it was reinseminated with semen from the same bull. There was a total of 368 inseminations.

Animals were grouped for slaughter on two days so that fetuses would be about 60 to 90 days of age at the time of slaughter. One week before scheduled slaughter the heifers were checked for pregnancy by examination of the reproductive organs per rectum. There were 111 animals diagnosed as pregnant. All 111 animals were examined at slaughter and at that time ranged from 63 to 88 days from the last insemination. Animal identification numbers accompanied the reproductive organs as they were removed from the cows to identify the source of the fetuses.

The ovaries, reproductive tracts and fetuses were examined carefully. The fetuses were weighed to the nearest gram and the crown-rump length measured to the nearest millimeter.

Analysis of variance, correlation and regression analyses were applied to the data. Bulls and sex of fetus were fixed and age of fetus was a random variable.

^aLutalyse, The Upjohn Co., Kalamazoo, MI 49001

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RESULTS

There were 104 normal fetuses recovered from 111 females, all singles. The sex ratio was normal. In addition there was one small degenerating fetus with grossly normal features. Two cows had reproductive tracts with small caruncles undergoing atrophy. Four heifers had no direct indications of pregnancy, although there were corpora albicans on the ovaries. Three of the fetuses were substantially larger than expected. The size corresponded to the expected size, if the females conceived at the previous insemination. There were 96 fetuses for which the length and weight were recorded. All appeared to be completely normal. Results are summarized in Table 1. There was no difference in the size of the fetuses sired by

Table 1. Length and weight of the fetuses at slaughter.

Sex of fetus	Item	Fetuses by bull A			Fetuses by bull B			Overall bulls		
		No. ^a	Mean	SD	No.	Mean	SD	No.	Mean	SD
Male	Age, days	25	78.4	+ 7.5	27	78.0	+ 8.8	52	78.2	+ 8.1
	Wt., grams	25	107.8	+ 54.9	27	112.9	+ 67.3	52	110.4	+ 61.1
	Length, cm.	25	13.4	+ 2.4	27	13.2	+ 3.1	52	13.3	+ 2.8
Female	Age, days	25	77.4	+ 8.4	17	75.6	+ 8.8	42	76.7	+ 8.5
	Wt., grams	25	92.2	+ 47.5	17	89.1	+ 59.0	42	90.9	+ 51.8
	Length, cm.	25	12.5	+ 2.5	17	12.2	+ 2.7	42	12.4	+ 2.25
Male and female	Age, days	50	77.9	+ 7.9	44	77.1	+ 8.8	94	77.5	+ 8.3
	Wt., grams	50	100.0	+ 51.4	44	103.7	+ 64.6	94	101.7	+ 57.7
	Length, cm.	50	12.9	+ 2.4	44	12.8	+ 3.0	94	12.9	+ 2.7

^a Number of fetuses measured in each group. Two fetuses presumably from earlier breedings, were 101 days old and weighed 478 and 450 grams, respectively. They measured 23 and 22 cm. in length. Both were male fetuses sired by Bull B. Since these 2 older fetuses would have distorted the means in the male group associated with Bull B, they were excluded, and only 94 are represented in the table. The third older fetus did not have a weight recorded.

the two bulls. Averaged over sexes the weights of fetuses sired by A and B were 100.0 versus 103.7 grams and the lengths were 12.9 versus 12.8 cm. Over the whole time covered, female fetuses tended to be smaller than the male fetuses. Average lengths of the males and females were 13.3 versus 12.4 cm and corresponding weights were 110.4 and 90.9 grams.

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Initially, regressions for each sex of fetus for each bull were fitted for length of fetus on age, and weight of fetus on age. All of the individual regressions indicated that age accounted for most of the variation in length and weight. All R^2 values were from 0.92 to 0.96. So the data for the two bulls were combined. The regression of length of fetus (Y), in cm, on age of fetus, in days (X), was $Y = -12.61 + 0.3308X$ for male fetuses. The regression for female fetuses was $Y = -9.453 + 0.2847X$. The R^2 values for males was 0.95 and for females was 0.92.

The curvilinear regression equations and associated lines for fetal weights on age are given in Figure 1. Individual values were close to the lines and overlapping, and are omitted for clarity. The good fit is indicated by the high R^2 values. It is obvious from Figure 1 that the male and female fetuses were diverging in weight as they developed.

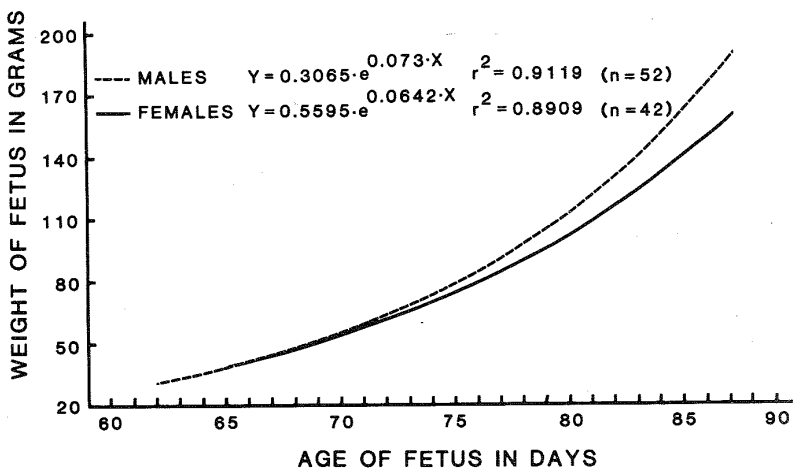


Figure 1. Curvilinear regression equations fitted to weights of male and to female fetuses. The original data points were so overlapping along these lines that they are omitted, but the goodness of fit is indicated by the high R^2 values.

An overall relationship between fetal age and both crown-rump length and fetal weight is illustrated in Figure 2. The two isolated open circles on the graph are mean values reported by Hafez and Rajakowski (2) for 90-day old Hereford fetuses (length 15.9 cm, weight 157 gr).

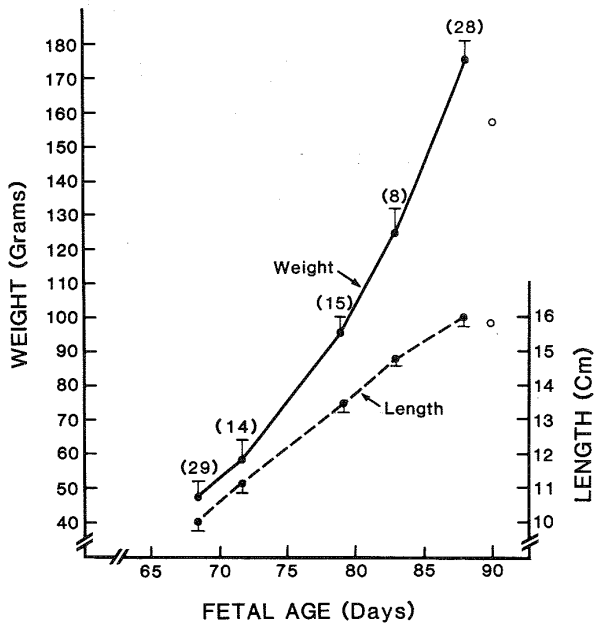


Figure 2. Length and weight of fetuses (means + standard errors) grouped by five-day intervals of age. Means do not occur at the mid-points because of fetal age distributions. Number of fetuses are in parentheses.

DISCUSSION

The larger size of the male fetus is consistent with other reports (1-3) and is consistent with the generally larger size of the male calves at birth. With the relatively large number of fetuses under highly controlled conditions it was possible to demonstrate that this difference was already significant before 90 days of gestation. Sires did not affect fetal size differently. This was not surprising because market survey records kept by Eastern A.I. Coop., Inc. on calving ease indicated that both sires were near the Holstein breed average in this regard, suggesting that calves sired by the two bulls tended to be similar in size. Overall the fetal size in this study generally parallels the variable values given for a few Holsteins during the same time period by Swett *et al.* (3). The values are slightly larger than those reported for smaller dairy breeds (3) or for Herefords (2).

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The high correlations between fetal size (length and weight) and age support the validity of estimating fetal age by careful palpation of the fetus per rectum at this time. However, the standard errors (Figure 2) indicate that the variation is sufficient to prevent precise prediction of fetal age by this method.

There were 3, or about 3%, of the animals which almost certainly had conceived at a previous insemination and pregnancy continued. Inseminations were performed by a professional inseminator who did not attempt to fully penetrate the cervix of females inseminated previously. This practice is followed to avoid disturbing a possible early pregnancy, as it is well-known (5) that a small portion of heifers and cows exhibit standing estrus during early pregnancy.

There were another 4% which had been recently diagnosed as pregnant, but in which there was no evidence of pregnancy. The reasons for this discrepancy are unknown.

These studies support the conclusion that the special processing of the semen did not affect the fetuses at 63 to 88 days, based upon their general appearance, length and weight. The two sires which likely produced calves of similar size also produced young fetuses of similar size. Only sex of fetus significantly affected growth rate.

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