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In two cattle populations; dairy and beef, the relationship between a bull's average spermatozoan midpiece length and production traits of economic importance was studied. In the first population involving 39 Holstein sires, variation in midpiece length between bulls and between ampules of semen from the same bull collected at least six months apart was highly significant ($P \leq .01$). Differences in midpiece length between ampules of semen prepared from the same ejaculate were negligible. From two methods of computation, heritability of midpiece length was estimated to equal (1.09 ± 1.03 and 1.30 ± 1.13. Phenotypic correlations between sire midpiece length and daughter's average dairy production traits were low to moderate but were consistantly negative. Phenotypic correlations between sire midpiece length and his own merit for semen quality and fertility traits were close to zero. This study suggested that midpiece length might be heritable and might be correlated with economically important production traits in dairy cattle.

In the second population involving 80 yearling Hereford bulls (17 Polled Hereford and 63 Horned Hereford), variation within bulls

for midpiece length was small (average coefficient of variation = 2.5%). Age did not significantly affect a bull's average midpiece length but the breed difference was highly significant ($P \leq .005$) with Polled Hereford bulls exceeding Horned Hereford bulls. Heritability of midpiece length estimated from paternal half-sib analysis of variance was 0.84 ± 0.56. Other estimates of heritability were computed for reproductive and growth traits and were found to be higher than values reported in the literature, perhaps due to a ranch of origin and/or pen effects. In general, phenotypic correlation between bull average midpiece length and his own reproductive and growth traits were low. Genetic correlations between average midpiece length and growth and reproductive traits were zero to moderate, and all were accompanied by large standard errors. This study suggested that midpiece length in Hereford cattle is highly heritable although further research is needed involving more suitable beef cattle populations to determine if midpiece length is correlated genetically with traits of economic importance.

THE INHERITANCE OF SPERMATOZOAN MIDPIECE LENGTH AND ITS RELATIONSHIP WITH TRAITS OF ECONOMIC IMPORTANCE IN CATTLE

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INTRODUCTION

In animal breeding, an economical trait of interest can be altered genetically through direct, indirect or index selection. When an animal is selected on the basis of its own phenotypic merit for the trait of interest, direct selection has been practiced. In urilizing single trait direct selection, the heritability of the trait is the only genetic information of interest. Alternatively, a trait can be altered through selection emphasis on a second trait, i.e., indirect selection. This is possible when the second trait is heritable and is genetically correlated with the economic trait of interest. With indirect selection, the heritability of both traits as well as the genetic correlation between them is needed to predict selection response. Assuming that the traits are genetically correlated, the economic trait of interest can be improved at a more rapid rate when a combination of both direct and indirect selection is practiced. For index selection combining information from both traits, the heritabilities of both traits and the genetic and phenotypic correlations between them are needed.

In mice, Beatty (1969) found a positive relationship between spermatozoan midpiece length (MPL) and body weight at maturity. Among four divergent lines, a 0.2 μm increase in MPL was associated with a 10 gram increase in body weight. Beatty hypothesized that an increase in mitochondrial content (synonymous with an increase in MPL) might cause a higher rate of oxidative phosphorylation which could affect the

manifestation of a wide array of energy dependent characters (e.g., body weight). Estimates of heritability for MPL in mice are high; 0.76 ± .02 (Woolley, 1970b) and 0.97± .36 (Woolley and Beatty, 1967).

Primarily because of the above mouse experiments, this work was initiated. In two cattle populations, dairy and beef, the biological relationship with certain economic traits and the heritability of spermatozoan midpiece length will be investigated and reported.

REVIEW OF LITERATURE

Spermatogenesis and Spermiogenesis

Spermatogenesis is the process by which sperm cells develop in the testes of the male. In meiosis I of spermatogenesis, a primary spermatocyte divides by meiosis to form two secondary spermatocytes. The significance of this process is the reduction of chromosomal material from the diploid to the haploid number (in cattle $2n = 60 \rightarrow n = 30$). In meiosis II, each secondary spermatocyte divides mitotically to produce two nonmotile spermatids (see Figure 1). During spermiogenesis, the metamorphosis of each nonmotile spermatid into a viable spermatozoan requires approximately fifteen days (Hafez, 1975, p. 101-122). Migration of centrioles, Golgi apparatus, mitrochondria and other cytoplasmic entities to specific regions on the spermatid also takes place during spermiogenesis (see Figure 2).

Migration of mitochondria in the cytoplasm of the spermatid to the proximal region of the flagellum (tail of the sperm cell) occurs during spermiogenesis. The quantity of mitochondria found in this

region is determined by the deposition of a fibrous periflagellar sheath on the main-piece of the early spermatid (Yasuzumi, 1956: Sotelo and Trujillo-Cenoz, 1958). There is some evidence that there are more mitochondria found in the cytoplasm of the spermatid than are incorporated in the midpiece (Bishop and Walton, 1960). Migrating to opposite ends of the midpiece are the proximal and distal centrioles (Lommen, 1967). It is after deposition of the periflagellar sheath and the migration of the centrioles that mitochondria conjugate on the midpiece.

After a brief period of turgidity and contortion, mitochondrial units align themselves and transform to an α-helical configuration, resembling the structure of the DNA molecule (Woolley, 1970). Woolley (1970) also reported that differences between individual sperm cells in MPL were associated with differences in the number of spiral units or gyres. Furthermore, it was calculated that each mitochondrion occupied 0.58 of one gyre. Thus, differences in MPL between cells are attributable to differences in mitochondrial content. Significant variation in MPL among males has been well documented in mice (Woolley 1970; Beatty, 1970, 1971; Sharma, 1960). On the spermatozoan midpiece of bull, man and mouse, approximately 10, 10 and 350 mitochondria are found (Fawcette, 1975).

The Role of the Mitochondria

The mitochondrion is the primary site of energy transformation in all eukaryotic organisms. The enzymatic systems controlling the

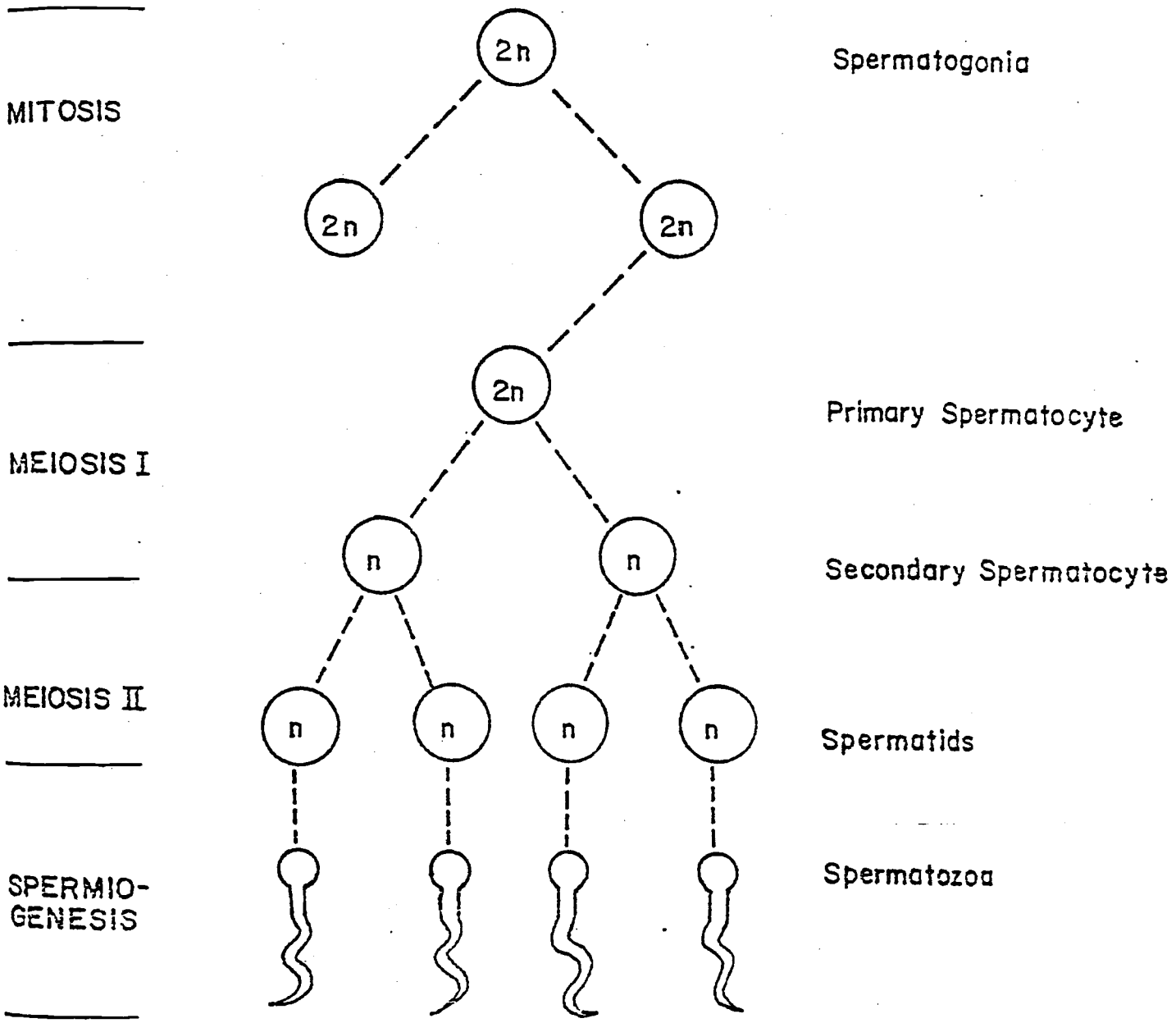

FIGURE 1. Spermatogenesis .

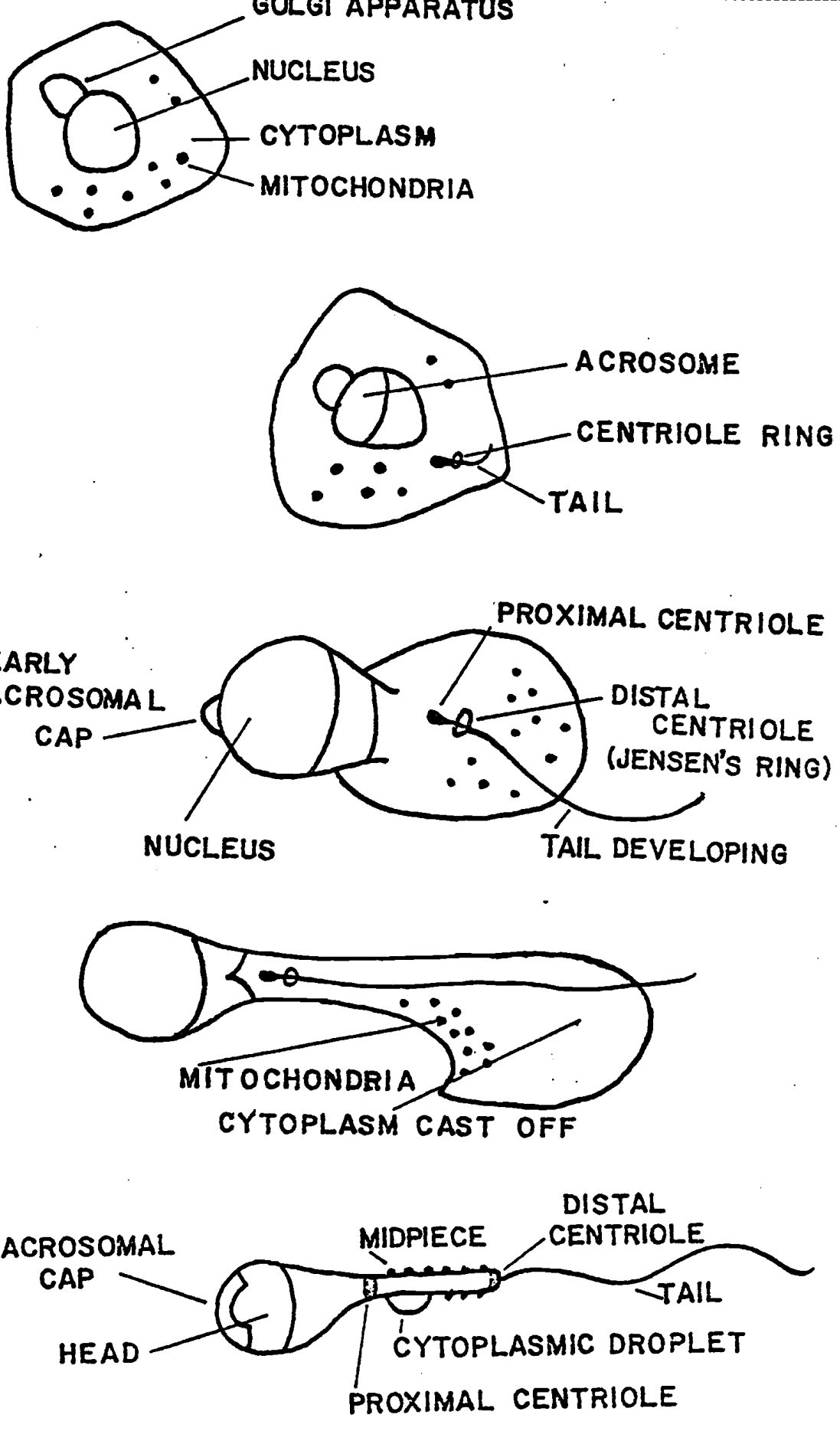

FIGURE 2. Spermiogenesis (Adaptation of Hafez, 1975).

Krebs Cycle, oxidative phosphorylation and the electron transport chain all occur in the inner membrane of the mitochondrion matrix (Lehninger, 1973, p. 509-542). It has been established that the mitochondria possess their own DNA and RAN (Wagoner, 1972), the DNA strands existing in closed circles measuring about 5 μm in length (Borst, 1970). Furthermore, nuclear and mitochondrial DNA coded proteins may interact during cellular metabolism (Woodward, et al., 1970). More specifically, cytochrome oxidase, a dimeric enzyme found in the mitochondrion and active in electron transport, has subunits whose amino acid sequences are coded by mitochondrial and nuclear genes (Chen and Charalampous, 1969).

Bi-parental contribution of mitochondria to the zygote at fertilization may occur. Under electron microscopy, Soupart and Strong (1974) and Zamboni, et al. (1972) observed in human oocytes that maternally and paternally derived mitochondria were present at fertilization. On the contrary, Yasuzumi (1956) observed that in the oocyte of the rat, degeneration of paternally derived mitochondria (on the spermatozoan midpiece) took place within a matter of hours of fertilization.

Polymorphism at mitochondrial loci has been hypothesized as an underlying cause of differential respiration rates between lines of mice (Wagoner, 1972), maize (Sarkissian and Srivastava, 1967) and Drosophila (McDaniel and Grimwood, 1971). The interaction between isolated mitochondria from parental types or strains in rate of respiration has been used as a test for predicted parental combining

ability and actual heterois (McDaniel and Sarkissian, 1966; McDaniel and Grimwood, 1971; Sarkissian and Srivastava, 1967). Lints and Lints (1967, 1968, 1969), however, were unable to detect a strong correlation between respiration rate and metric characters in Drosophila.

The Relationship between Midpiece Length and Quantitative Traits

In mice, Beatty (1969) reported the existence of a positive phenotypic relationship between spermatozoan midpiece length and mature body weight. He found that for every 0.2 μm increase in MPL an increase of 10 grams in body weight was observed. Beatty advanced the hypothesis that an increase in MPL associated with mitochondrial content was related to a higher rate of oxidative phosphorylation and/or ATP synthesis. If this is true, it would be expected that a great number of other traits would also be affected.

High estimates of heritability for MPL in mice (0.97 ± .36, Woolley, 1970b) have been reported. Woolley (1970b) practiced bi-directional selection for MPL in mice and demonstrated a rapid rate of response for the trait. After thirteen generations of selection, the lines differed in MPL by 5.4 phenotypic standard deviations, and the responses were essentially symmetrical. One criticism of this experiment could be that no attempt was made to investigate the relationship between the change in mitochondrial content (midpiece length) and other quantitative, energy dependent characters. The relationship between MPL and fertility traits in mice was, however,

examined by Woolley (1970a). In that experiment, color coded males representing three MPL lines; high, unselected and low, contributed seminal collections. Samples were pooled, and heterospermic inseminations to an unrelated line of inbred females were made. Results showed no indication of differential fertilizing ability of spermatozoa between the three paternal lines. After the birth of over 500 offspring, the hypothesis that a relationship existed between MPL and fertility was rejected. Furthermore, Beatty and Mukherjee (1963) and Pant (1971) have reported that neither age in males nor maternal effects attributable to dams influenced MPL in mice.

In cattle, significant variation among breeds and among bulls within breeds in spermatozoan MPL has been reported (Mukherjee and Singh, 1965, 1966). Variation in MPL between seasons and/or collections and between slides within collections apparently are minimal (Mukherjee and Singh, 1965, 1966). No attempt was made by these workers to investigate the relationship between MPL and economically important production traits.

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CHARACTERISTICS OF SPERMATOZOAN MIDPIECE LENGTH AND ITS RELATIONSHIP WITH ECONOMICALLY IMPORTANT TRAITS IN CATTLE[1]

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Abstract

Statistical properties, inheritance of spermatozoan midpiece length, and its association with bull reproductive traits and daughters' dairy traits were examined in a population sample of 39 Holstein bulls. Variation for midpiece length of individual cells was small within bulls (coefficient of variation ≅ 4.5%), but variation for midpiece length did exist among bulls. Average midpiece length did not differ in ampules in semen collected from the same ejaculate, but differences did exist between ampules of semen collected from the same bull at least 6 mo apart. Two methods of estimation yielded heritabilities of midpiece length greater than one. Both were imprecise, but both were consistent with the existence of additive genetic variation for the trait. Moderate correlations between a sire's midpiece length and his predicted difference for dairy production traits were negative. Correlations between midpiece length and semen quality and fertility were close to zero. Results suggest that midpiece length is heritable and that it might be correlated with economically important dairy production traits.

(Key words: Sperm midpiece length, cattle, production, reproduction.)

INTRODUCTION

Progeny testing can be used to estimate a sire's genetic merit for lowly heritable traits and for female sex-limited traits such as milk production. An alternate procedure would be to estimate breeding value for the sex-limited trait from the sire's phenotypic merit for another trait, provided the second were highly correlated genetically with the first and were highly heritable (24). In chickens, such a parallel relationship may exist between packed sperm volume and egg mass (9); and in mice and sheep, testes size and ovulation rate are positively genetically correlated (7,8).

In mice, a positive relationship between spermatozoan midpiece length and body weight at 13 to 17 wk of age has been reported (1). Since the midpiece is composed of mitochondria surrounding the proximal region of the flagellum in an α-helical configuration, it is possible that an increase in midpiece length associated with the quantity of mitochondria could be associated with a higher rate of oxidative phosphorylation (2). This in turn could be associated with a variety of other important traits. Variation in midpiece length in the mouse studies was attributable to a difference in mitochondrial content (22). The number of mitochondria per spermatozoan of bull, man, and mouse is approximately 10, 10, and 350 (4). Midpiece length in mice is highly heritable (.76 ± .02; .97 ± .36) but is not correlated with lowly heritable traits such as fertility (20,23,21). A general lack of age and maternal effects for midpiece length has also been reported (3,15).

Biologically, mitochondria are the energy transformers of the cell. Many important enzymes that are involved directly in the Krebs Cycle are synthesized according to mitochondrial DNA instructions (18). Nuclear and mitochondrial DNA coded proteins may interact throughout cell metabolism and growth (19). In Drosophila and maize, a close correspondence between actual heterosis and mitochondrial complementation has been demonstrated (11,16). More importantly, controversial evidence of mitochondrial duplication, mitochondrial recombination and division, and restoration of mitochondrial content following union of the sperm and ova has been reported (17,25).

Our objectives were to describe statistical properties of spermatozoan midpiece length (MPL) in Holstein bulls, to partition variation in MPL among bulls, between separate collections within bulls, and within collections, to examine the inheritance of MPL, and to measure correlations between MPL and fertility and dairy production traits.

EXPERIMENTAL PROCEDURES

To increase visibility of the midpiece region, thawed semen was diluted 1:15 with physiological saline phosphate buffer solution, fixed in osmium tetroxide (4% aqueous) for 10 min, stained for 1 h in Harris' Alum Hematoxylin solution, counterstained in a 2.5% solution of ferric alum for 10 min, and differentiated in picric acid for another 10 min, an adaptation of the method of Hancock (6). Under light microscopy, spermatozoan MPL was measured with a rotometer.

To ensure that MPL was being measured consistently, in a pilot trial three repeat measurements of MPL were made for each of 100 sperm cells from each of two Holstein bulls. Computation of sample variance and repeatability of MPL (for each bull) allowed calculation of the required number of cells to measure per bull (McClave and Dietrich, 10, p. 228) and the required number of repeat measurements per cell for accurate determination of a bull's average MPL.

A second pilot trial was conducted to ensure that variation for average MPL was minimal between ampules from the same ejaculate (variation due to micro-environmental influences). Twenty-five cells were measured for MPL from each of two ampules from each of three Holstein bulls. Data were analyzed by heirarchical analysis of variance.

In the main experiment, 39 Holstein bulls were chosen at random from the battery of American Breeders Service, DeForest, WI (with stipulations that their repeatability for Predicted Difference for milk exceeded .60 and that semen from each bull was available from two collections at least 6 mo apart). Twenty-five cells were measured for MPL per ampule per bull. These data were analyzed by hierarchical analysis of variance to partition variation in MPL to differences among bulls, differences between collection periods (at least 6 mo apart) within bulls, and differences within ampules within bulls.

To study the inheritance of MPL, the symmetric differences squared method of Grimes and Harvey (5) was used, with the assumption that maternal effects of MPL were not important. For each of the 741

possible pairs of bulls ((39 x 38)/2), the squared difference for average MPL was computed. The genetic relationship (R) between each pair of bulls also was computed. Based upon the assumption that the genetic relationship of the particular pair of bulls and upon genetic variation for MPL in the population, the model

$$\frac{(MPL_i - MPL_j)^2}{2} = V_E + (1-R)\ V_A \qquad (1)$$

was solved by regression methods. MPL_i and MPL_j are average midpiece lenghts for pairs of bulls ($i \neq j$), V_E is residual variation, V_A is additive genetic variation and R is genetic relationship between the pair of bulls in question. In comparison to the standard linear regression model:

$$Y = a + bX, \qquad (2)$$

half the squared difference for MPL corresponds to Y, 1-R corresponds to X, a is an estimate of V_E for MPL in the population of interest and V_A corresponds to the regression coefficient b. Heritability of MPL was estimated as the ratio of V_A to $V_A + V_E$. For comparison, heritability also was estimated by paternal half-brother analysis. The mathematical model included only sires of bulls and the residual term as sources of variation. The standard error of heritability was computed according to the approximation formula of Osborne and Patterson (14). Finally, product-moment correlations were computed between MPL and fertility and dairy production traits on which data were available from American Breeders Service and official 1979 USDA sire summaries, respectively.

RESULTS AND DISCUSSION

For three measurements of MPL for each of 100 cells from two bulls, repeatabilities were .66 and .74. We therefore decided only one measurement per sperm cell would be made in subsequent studies. The decision to measure 25 cells per bull was based upon statistics from this preliminary trial (Table 1).

Results in Table 2 indicate most importantly that differences in average MPL between ampules collected from the same ejaculate were small, accounting for only 2% of the variation. Our purpose in conducting this trial was to ensure that micro-environmental factors did not affect MPL significantly. Mukherjee and Singh (12) also reported little difference in MPL between slides within collections within three dairy breeds of bulls.

Average MPL for all bulls was 13.41 μm with a standard deviation of .43. Statistically significant differences did exist among the 39 bulls (Table 3). Mukherjee and Singh (12) also reported differences among sires in average MPL, while Mukherjee and Singh (13) reported significant among-breed differences as well. Differences in average MPL between ampules of semen collected at least 6 mo apart were significant, accounting for 14% of the phenotypic variation. However, the correlation between a bull's average MPL in the two collections exceeded .80. In the experiments of Mukherjee and Singh (12,13), variation in MPL from seasonal and between-collection differences was not significant.

Some 85% of paired combinations of individuals from the 39-bull

population sample were not related genetically. Relationships between the remaining pairs ranged from 3.125 to 31.125%. In Table 4, the average difference in MPL is shown for each genetic relationship. Although the difference in MPL and R were not linearly related, there was some tendency for the MPL difference to be smaller between related than between unrelated pairs of bulls, as would be expected if MPL were heritable.

By using the symmetric differences squared method (5), the following linear model was obtained:

$$\frac{(MPL_i - MPL_j)^2}{2} = -.038 + (1-R).165, \qquad (3)$$

where -.038 and .165 are estimates of environmental and additive genetic variance.

Heritability for MPL was:

$$h^2 = \frac{.165}{-.038 + .165} = 1.30 \pm 1.13 \qquad (4)$$

Heritability of MPL from paternal half-brother analysis, based upon eight paternal half-brother families of two to five bulls each, was 1.09 ± 1.03. Both the traditional method and the symmetric differences squared method yielded imprecise estimates of heritability because of limited data and for the latter method the preponderance of unrelated pairs of bulls. Both estimates, however, are consistent with the existence of additive genetic variation for the trait.

As shown in Table 5, daughter dairy traits were not highly correlated with a sire's average MPL (based upon 25 cells from each of two

collections taken at least 6 mo apart). These correlations are neither genetic nor phenotypic, in the commonly accepted sense, since they relate MPL, measured directly on the bulls, to production traits measured on variable numbers of daughters. Magnitude and sign of the correlations would be a function of the genetic correlation between MPL and the dairy trait of interest as well as .50 (accounting for the Mendelian segregation between sire and daughter), square roots of heritabilities of MPL and dairy trait, and the number of daughters. For the correlations which are negative, it can be inferred that the genetic correlation, if real, must also be negative; and sires with larger than average MPL would tend to produce daughters below average for dairy production traits.

Correlation coefficients between MPL and semen quality and fertility traits were all near zero (Table 6). In addition, the correlation between a sire's average MPL and the percentage of his daughters culled during their first lactation was only .15 (Table 5). Thus, there was no evidence of association between MPL and fitness traits.

The 39 Holstein bulls in our population sample all had highly repeatable Predicted Differences for dairy traits, and all were in use at a commercial artificial insemination organization. Thus, they were a random sample of all Holstein bulls neither for dairy nor for fertility traits. Whether they constituted a random sample with respect to spermatozoan MPL is dependent upon the correlation, in the original unselected population, between MPL and the traits upon which selection of the bulls for extensive A.I. use was based.

Work currently is underway to investigate variation among bulls for MPL and to compute heritabilities and genetic and phenotypic correlations between MPL and economically important traits in two beef cattle populations not subject to these limitations.

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TABLE 1. Statistical properties of midpiece length based upon the first of three measurements per cell

	Bull 1	Bull 2
Sample size	100	100
Mean (μm)	13.30	13.63
Variance	.41	.36
Coef. of variation	4.8%	4.4%

TABLE 2. Hierarchical analysis of variance for differences among bulls and between ampules for midpiece length

Source	df	MS	Variance component
Bulls	2	9.73**	.188
Ampules/Bulls	3	.33	.007
Cells/Ampules/Bulls	144	.15	.146

**$P \leq .01$

TABLE 3. Hierarchical analysis of variance for differences among bulls and between collections for midpiece length

Source	df	MS	Variance component
Bulls	38	6.16**	.096
Collections/Bulls	39	1.35**	.047
Cells/Collections/Bulls	1,872	.19	.186

**$P \leq .01$

TABLE 4. The association between genetic relationship and MPL difference for all possible pairs of bulls

Genetic relationship (%)	Number of pairs	Average difference in MPL (μm)
0	632	.41
3.13	5	.20
6.25	38	.28
12.50	34	.45
25.00	29	.31
28.13 - 31.13	3	.31

TABLE 5. Product-moment correlations between sire midpiece length and daughters' dairy traits

Trait	r
Predicted difference for milk yield	-.28
Predicted difference for butterfat percentage	-.04
Predicted difference for butterfat yield	-.30
Predicted difference for dollars	-.38*
Percent of daughters culled during first lactation	.15

*$P \leq .05$

TABLE 6. Product-moment correlations between sire midpiece length and semen quality and fertility traits

Trait	r
Motility at time of collection	-.02
Percent abnormal cells	-.05
Post-thaw motility	-.07
Monreturn rate	.02

THE INHERITANCE OF SPERMATOZOAN MIDPIECE LENGTH AND ITS RELATIONSHIP WITH GROWTH AND REPRODUCTIVE TRAITS IN HEREFORD CATTLE

S. D. Lukefahr, William Hohenboken and J. S. Brinks

SUMMARY

Semen samples were collected from 80 yearling Hereford and Polled Hereford bulls, and the relationship between average spermatozoan midpiece length (MPL) and growth and reproductive traits was examined. Variation within bulls for average MPL was small (c.v. = 2.5%). The effect of age was negligible in influencing average MPL, but breed effects were highly significant. Heritability of MPL estimated by paternal half-sib analysis was 0.84 ± 0.58. Phenotypic correlations between average MPL and several growth and reproductive traits were low. The magnitude of the genetic correlations between average MPL and growth and most reproductive traits were zero to moderate and were accompanied with large standard errors. These results suggest that MPL is highly heritable. Further investigation, however, in more suitable beef cattle populations is needed to determine whether MPL is correlated genetically with traits of economic importance.

INTRODUCTION

Indirect selection can be an effective method by which to alter a trait of interest in a desired direction. This is especially true when the indirect traits is highly heritable and is highly genetically

correlated with the trait of interest (Young and Turner, 1965).

In mice, a positive relationship between spermatozoan midpiece length (MPL) and mature body weight has been reported (Beatty, 1969). Beatty found that each 0.2 μm increase in MPL was accompanied by a 10 gram increase in body weight. He hypothesized that an increase in mitochondrial content (synonymous with increased MPL) might be related to a higher rate of oxidative phosphorylation and might thus affect energy dependent traits. In mice, variation among males in sperm MPL has been reported (Beatty, 1969; Pant, 1971). Woolley (1970c) ascertained that differences among males for MPL were due to differences in the number of mitochondria-containing spiral gyres surrounding the periflagellar membrane of the midpiece. Each mitochondrion was found to occupy 0.58 of one gyre. On the spermatozoan midpiece of bull, man and mouse, approximately 10, 10 and 350 mitochondria are found (Fawcette, 1975). Woolley and Beatty (1967) and Woolley (1970b) reported estimates of heritability for MPL in mice of 0.97 ± .36 and 0.76 ± .02, respectively. In addition, the influences of age and maternal effects on MPL were minimal (Beatty and Mukherjee, 1963; Pant, 1971); and the relationship between MPL and conception rate was small (Woolley, 1970a).

In the study reported herein, semen samples from 80 yearling Hereford and Polled Hereford bulls completing post-weaning gain tests at the San Juan Basin Experiment Station of Colorado State University were collected, prepared for microscopic examination and measured for MPL determination. Objectives of the study were: 1) to obtain

descriptive statistics for MPL and performance traits of bulls, 2) to investigate the effects of age, breed, and sires within breeds on average MPL and performance traits of the bulls, 3) to estimate the heritability of MPL and growth and reproductive traits and 4) to determine phenotypic and genetic correlations between average MPL and growth and reproductive traits.

MATERIALS AND METHODS

At the San Juan Basin Research Center, Hesperus, Colorado, of Colorado State University, 225 Hereford, Polled Hereford, Angus, Red Angus, Simmental, Limousine, Charolais, Red Brangus, Salers and Santa Gertrudis bulls were evaluated for post-weaning rate of gain on a 140-day test to approximately one year of age. For the study reported herein, data from 63 Hereford and 17 Polled Hereford bulls were utilized. Fifty-three Hereford and 7 Polled Hereford bulls were excluded because stained semen samples from them were not appropriate for sperm MPL determination.

Bulls were group fed in pens of five or six bulls each. In most pens, all bulls were from the same ranch; and in many pens, all bulls were paternal half-sibs. Therefore, sire effects in subsequent analyses were confounded to a degree with ranch and/or pen effects.

Traits examined were actual weaning weight, weaning weight adjusted for calf age and age of dam, initial weight on test, average daily gain on test, actual weight at the termination of the 140-day test and age adjusted yearling weight.

Shortly following the end of the test period, scotal circumference (Coulter and Foote, 1979), semen quality and a subsequent determination of average MPL were recorded. Semen traits included percentages of motility, abnormal heads, separated heads, abnormal midpieces, normal cells, abnormal tails, proximal droplets, distal droplets and dead cells.

Staining of sperm cells was necessary to improve the visibility of the midpiece region. Seventy of the 80 bulls had semen samples collected on April 15-17, 1980, and staining was accomplished using an adaptation of the method of Hancock (1952). Semen was smeared onto glass slides and allowed to air-dry. Slides were then fixed for 10 minutes throgh immersion in an osmium textroxide (4% aqueous) vapor bath, stained for 1 hour in Harris' Alum Hematoxylin solution and counterstained for 10 minutes in a 2½% ferric alum solution. After rinsing in distilled water and air drying, slides were ready for MPL determination under light microscopy. Seventy of 84 slides prepared using this technique were appropriate for MPL determination. Cells were measured for MPL with the use of a rotometer.

Semen was collected from the remaining bulls a few days later. The staining procedure for these bulls involved smearing fresh semen samples equally diluted in Hancock's solution (1 gm Eosin Y per 60 ml of 10% aqueous Nigrosine) across glass slides. After they were allowed to dry, slides were ready for determination of MPL under phase-contrast microscopy with the use of a rotometer. Of 70 slides prepared using this method, only 10 were appropriate for MPL determination. Poor

sperm concentration and cracking of the stain made most of the slides unusable for MPL determination.

To quantify the degree of variability for MPL, 100 cells were measured for each of two bulls sampled at random from within the population. Based upon the descriptive statistics for MPL from this preliminary work, the number of cells required to accurately estimate average MPL for the remaining bulls was determined.

In preliminary data analysis, staining method and/or type of microscopy was examined as a potential source of variation in average MPL. Samples prepared using the Hancock staining solution and examined under phase microscopy had MPL's consistently shorter than samples stained with the adapted method of Handock (1952) and examined under light microscopy. Therefore, average MPL of individuals that had been evaluated by Hancock staining/phase microscopy were adjusted to the expected modified Hancock staining/light microscopy equivalent by the multiplicative factor of 1.062.

Variation in average sperm MPL and in bull growth and semen traits was analyzed using a mathematical model including fixed sources of variation for age and breed and the random effect of sires nested within breeds. Analyses of variance utilized Harvey's LSML76 program. Estimates of heritabilities, genetic correlations and phenotypic correlations also were obtained from the analyses.

RESULTS AND DISCUSSION

From the preliminary examination of 100 cells from each of two randomly chosen bulls, coefficients of variation were each 3.0%, and the two bulls differed by 4.0% for average MPL. It was decided that the measurement of 25 cells per bull was sufficient for an accurate estimate of average MPL. The average within bull coefficient of variation for all bulls in our study was 3.5%.

Least-squares means and residual standard deviations for average MPL and for the growth and reproductive traits are presented in Table 1, with a summary of the analyses of variance results and estimated heritabilities of the traits.

Only the effect of breeds was significant in influencing average MPL. Least-squares means for average MPL for Herefords and Polled Herefords were 13.04 μm and 13.35 μm, respectively. Mukherjee and Singh (1966) detected significant variation among Indian dairy breeds for MPL. Although the effect of sires within breeds on MPL was not significant in this study, it accounted for a considerable proportion of observed variance, as evidenced by the high heritability of the trait. Significant variation among sires within dairy breeds was reported by Mukherjee and Singh (1965) and Lukefahr and Hohenboken (1981).

Age, breed and sires within breeds significantly affected several growth and reproductive traits. As expected, age affected actual weaning, initial and 140-day weights, but age did not affect reproductive trait performance. The breed effect was significant for all

growth traits except actual and adjusted weaning weights and average daily gain, with Herefords exceeding Polled Herefords. For some reproductive traits, breed differences were important. Herefords exceeded Polled Herefords for scrotal circumference and for percentages of abnormal midpieces and proximal droplets but not for percent abnormal heads.

Heritability of average MPL was estimated to be 0.84 ± 0.58. Lukefahr and Hohenboken (1981) reported heritability estimates for average MPL of Holstein bulls of 1.09 ± 1.03 and 1.30 ± 1.13. Heritability estimates for actual weaning weight, adjusted weaning weight and initial weight were unreasonably high. It is likely that the paternal half-sib correlations for those traits were biased by ranch effects; the 36 sire groups contributing to the analysis came from 23 separate ranches. Ranch effects could include the overall manifestation of differences in herd health, maternal productivity, nutritional program, climatic conditions and other effects. For 140-day weight and adjusted yearling weights, heritability estimates were nearly as high, which could have reflected a carryover of ranch effects or effects from paternal half-sibs being penned together. The heritability for average daily gain was higher (0.89 ± 0.58) than average values from the literature (Woldehawariat et al., 1977), perhaps due to the partial confounding of ranch and/or pen effects with sire groups.

The heritability estimate for scrotal circumference was 1.39 ± 0.54. Coulter and Foote (1979) reported a mean heritability of

0.67 ± 0.10, averaged over age of bull for the same trait. In our study scrotal circumference was not highly correlated phenotypically with growth traits, such as 140-day weight (0.31). Therefore, the high heritability was not likely a carryover effect of ranch and/or pen effects on body size, but ranch/pen effects could have influenced scrotal circumference directly. Estimates of heritability for semen traits ranged from low to high in magnitude and all were accompanied by large standard errors.

Phenotypic correlations between average MPL and growth and reproductive traits were low (Table 2). This is in agreement with results of Lukefahr and Hohenboken (1981), who reported that phenotypic correlations between MPL and semen and/or fertility traits in Holstein bulls were close to zero. Although low, significant correlations between average MPL and percentages of motility and normal cells were observed. Lukefahr and Hohenboken (1981) reported that average MPL of Holstein bulls was moderately negatively correlated with predicted differences of their daughters for dairy traits.

Due to the small number of bulls (2.1) per half-sib family, genetic correlations between avarage MPL and growth and reproductive traits were all associated with large standard errors (Table 2). Determination of whether econmically important beef production traits are genetically correlated with average MPL will therefore have to await further investigations. Such research involving other beef cattle populations is currently underway.

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TABLE 1. Least-Squares Means, Residual Standard Deviations, Effects of Age, Breed and Sires Within Breeds and Heritabilities of Average MPL and Growth and Reproductive Traits.

Trait	Least-Squares Mean	Residual Standard Deviation	Age	Breed	Sires/Breed	h^2[a]
Average MPL (μm)	13.19	0.27	--	***	--	0.84 ± 0.58
Actual Weaning Weight (kg)	234.4	19.1	***	--	***	1.57 ± 0.52
Adjusted Weaning Weight (kg)	237.1	20.8	--	--	***	1.90 ± 0.48
Initial Weight on Test (kg)	258.0	19.1	***	***	***	1.78 ± 0.50
140-day Weight (kg)	430.7	28.7	*	**	**	1.45 ± 0.54
Average Daily Gain (kg)	1.23	0.28	--	--	--	0.89 ± 0.58
Adjusted Yearling Weight (kg)	405.8	27.4	--	**	*	1.32 ± 0.55
Scrotal Circumference (cm)	32.9	1.83	--	*	*	1.39 ± 0.54
Motility (%)	54.5	10.9	--	--	--	--[b]
Abnormal Heads (%)	4.92	2.97	--	***	--	0.68 ± 0.60
Separated Heads (%)	8.01	5.73	--	--	--	0.19 ± 0.61
Abnormal Midpieces (%)	6.03	4.60	--	***	--	0.73 ± 0.60
Normal Cells (%)	71.4	11.7	--	--	*	1.38 ± 0.55
Abnormal Tails (%)	5.19	6.17	--	--	*	1.08 ± 0.58
Proximal Drouplets (%)	2.23	3.28	--	*	--	0.24 ± 0.61
Distal Droplets (%)	1.80	2.87	--	--	--	0.23 ± 0.61
Dead Cells (%)	0.44	0.74	--	--	***	2.11 ± 0.45

*P ≤ 0.05
**P ≤ 0.01
***P ≤ 0.05

[a]Estimated by paternal half-sib analysis involving 36 families with an average sub-class size of 2.1 bulls.

[b]A negative estimate of heritability for percentage motility was obtained.

TABLE 2. Phenotypic and Genetic Correlations Between Average MPL and Growth and Reproductive Traits.

Actual Weaning Weight	80	-0.17	0.37 ± 0.43	Scrotal Circumference	80	0.07	-0.39 ± 0.48
Adjusted Weaning Weight	80	-0.20	0.45 ± 0.41	Motility (%)	79	0.29*	--[a]
Initial Weaning Weight	80	-0.17	0.33 ± 0.41	Abnormal Heads (%)	79	0.02	0.45 ± 0.56
Average 140-day Weight	80	-0.09	0.15 ± 0.44	Separated Heads (%)	79	-0.16	1.52 ± 1.73
Average Daily Gain	80	0.04	-0.25 ± 0.55	Abnormal Midpieces (%)	79	-0.07	-0.05 ± 0.55
Adjusted Yearling Weight	80	-0.13	0.36 ± 0.47	Normal Cells (%)	79	0.24*	0.04 ± 0.41
				Abnormal Tails (%)	79	-0.16	-0.13 ± 0.48
				Proximal Droplets (%)	79	-0.19	-1.08 ± 2.07
				Distal Droplets (%)	79	-0.08	-1.39 ± 2.44
				Dead Cells (%)	79	0.20	-0.43 ± 0.37

* $P \leq .05$

[a] rg was not computed due to a negative estimate of heritability for percentage motility

APPENDIX 1. Average MPL, variance of MPL, percent repeatability and predicted differences for several milk production traits for each of 39 Holstein bulls.

Bull I.D. Number	Average MPL	Variance of MPL	Repeat-ability (%)	Milk (lb.)	Fat (%)	Fat (lb.)	Difference
2980	13.23	0.24	97	+977	-.13	+16	+78
3220	13.67	0.21	63	-094	+.10	+10	+07
2810	13.42	0.16	66	+124	+.07	+15	+27
2910	13.10	0.32	60	+595	-.06	+13	+52
2475	14.14	0.19	98	+294	+.04	+16	+38
2628	13.18	0.16	73	+940	-.21	+03	+59
2546	13.71	0.12	84	+240	-.05	+02	+17
2668	13.20	0.19	73	+755	+.02	+31	+84
2766	13.70	0.17	60	+561	-.04	+15	+52
2818	12.97	0.08	68	+796	-.10	+14	+65
2820	13.57	0.16	82	+675	-.08	+13	+57
2576	13.14	0.12	79	+596	-.09	+09	+47
2691	13.40	0.12	73	+1036	-.17	+12	+77
2554	13.71	0.16	88	+477	-.06	+09	+40
2728	13.67	0.15	74	+406	+.04	+21	+51
2465	13.73	0.15	98	+1564	-.31	+09	+104
2389	13.11	0.09	98	+735	-.04	+21	+70
2636	14.19	0.34	69	+868	-.18	+05	+58
2781	13.47	0.18	62	+437	+.03	+20	+51
1958	13.24	0.15	99	+757	-.24	-08	+35
2588	13.17	0.17	73	+647	+.05	+31	+78
1953	13.27	0.21	98	+236	+.24	+43	+69
2662	13.15	0.17	69	+789	+.05	+36	+93
2890	13.10	0.28	93	+488	+.03	+21	+53
3280	13.39	0.10	81	+761	-.21	-04	+40
2825	13.45	0.17	85	+1096	-.18	+13	+82
2831	12.65	0.14	74	+1034	-.10	+22	+89

APPENDIX 1. (Continued)

Bull I.D. Number	Average MPL	Variance of MPL	Repeat-ability (%)	Milk (lb.)	Fat (%)	Fat (lb.)	Difference
2845	13.19	0.10	73	+582	-.11	+05	+41
2758	12.92	0.28	73	+1780	-.03	+60	+182
2864	12.97	0.16	66	+1074	-.18	+12	+79
2807	14.20	0.26	67	+495	-.14	-03	+25
2685	13.16	0.32	96	+676	-.15	+03	+44
2665	13.43	0.12	70	+834	+.02	+33	+92
2798	13.26	0.13	63	+895	+.01	+34	+97
2913	13.31	0.25	91	+310	+.19	+38	+67
2744	13.72	0.22	72	+960	-.05	+27	+91
2680	13.75	0.27	63	+816	+.03	+34	+84
2875	13.42	0.13	74	+973	-.03	+31	+97
2885	13.83	0.17	78	+732	-.13	+07	+52