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Ovarian Characteristics and Reproductive Performance of the Aged Cow[1]

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ABSTRACT

The reproductive performance of 152 grade Hereford cows that were 14 to 15 years of age at either death or slaughter resulted in an observed mean of 9.3 ± 0.3 calves and a calculated mean lifetime reproductive capacity of 10.4 calves. The median was 10 calves and the modal number was 12. As dictated by the criterion "failure to bear a calf during two successive years," 15 years was the age at which over 50 percent of the herd became infertile.

Ovaries from 69 fertile and 78 infertile cows were serially sectioned and microscopically examined; the groups did not differ in total germ-cell endowment ($P>0.05$), and fertile significantly exceeded infertile only in number of growing follicles ($P<0.01$). No intergroup difference occurred in either number or quality of vesicular follicles. The mean germ-cell endowment of 14- to 15-year-old cows was 24 ± 3 thousand (N = 147). Cystic corpus luteum was the apparent principal cause of infertility.

INTRODUCTION

Although published reports provide some insight into what may be the upper limit of the lifespan and reproductive capacity of the bovine female (Hammond and Marshall, 1952; Kohli and Suri, 1957), to our knowledge data that would provide a reasonable estimate of mean age at infertility and mean reproductive capacity have not been published. Also, reports contrasting the ovarian characteristics of the fertile and infertile aged bovine are not extant.

Data essential to these needs have been made available through a study designed to determine the long-term effects of γ-radiation on reproductive performance and germ-cell survival (Erickson et al., 1976). Results of that study did not reveal an irradiation effect on these criteria or on any other criterion tested ($P>0.05$). It therefore seemed reasonable to conclude that results of analyses reported herein are a close approximation of what could be expected from similar analyses of a nonirradiated population.

MATERIALS AND METHODS

Two groups of 140 grade Herefords that were 15 to 18 months of age upon entering the study were treated with either zero or varying levels of γ-radiation (200–600 R) during each of the years 1960 and 1961. Survivors were used to form a herd of 219 cows that was maintained until 1974. With the exception of five infertile animals slaughtered during the third year of the study, there was no culling. However, by 1973, when herd disposal was initiated, 67 cows had died as a consequence of a variety of causes (principal among which were tetany, bloat, dystocia, and cancer eye). Cause and rate of death were apparently unrelated to irradiation, but as a result of death the herd was reduced to 147 animals. Fifty-five infertile cows were slaughtered in 1973, and the study and the remaining cows were terminated in 1974 when 50 percent of the herd was infertile. First births occurred in 1962 and the last pregnancies occurred in 1974; hence, at a maximum the study allowed 13 pregnancies. Comparisons of the ovarian characteristics of fertile and infertile cows were limited to those that comprised the herd between the years 1973 and 1974 or cows that were 14 to 15 years of age at either slaughter or death (N = 147). For evaluations of reproductive performance, however, the five infertile animals mentioned above were included in the analysis, thereby increasing "N" to 152.

Throughout the experiment a bull-to-cow ratio of 1:25 was maintained. Bulls had access to cows for a 3-month period each year. A cow was judged infertile if she failed to deliver a calf during two successive years—a criterion found to be accurate 90 percent of the time. Of 77 cows observed through three or more years of apparent infertility, only 7 bore calves after the second nonproductive year. Uteri and oviducts were examined at slaughter and in only two cases were these organs the apparent cause of infertility. Ovaries were recovered at slaughter, fixed in Bouin's fluid, and serially sectioned at 10μ; every 20th section was mounted. Total ovarian endowment of primordial (oocyte encompassed by a single layer of follicle cells)

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and growing (oocyte with two or more layers of follicle cells in the absence of a fully formed vesicle) follicles was estimated from microscopically aided counts of every 60th section of both ovaries. Total vesicular follicles were estimated from an evaluation of every 200th section. Since the vesicular follicles were visible to the unaided eye, duplication in counting was readily avoided. Studies of sections mounted in a complete series revealed that counts of growing follicles would be accurate if the follicle were counted only in that section containing the oocyte nucleolus. By the same technique we found that accuracy of primordial-follicular counts would be enhanced if a follicle were counted only in that section containing a full cross section of the oocyte nucleus. Significance of group differences was assessed through an analysis of variance.

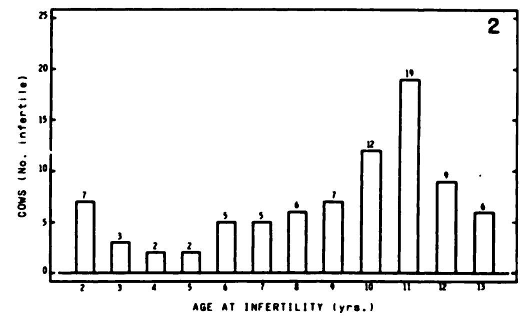

FIG. 2. Distribution of 83 Hereford cows with respect to age at infertility. Number above bars is number of cows.

RESULTS

The distribution of 152 grade Herefords according to number of calves produced during a period of 12 to 13 years is shown in Fig. 1. Only 12.5 percent of the population produced 5 calves or less. The median was 10 calves and the mean was 9.3 ± 0.3; a total of 1406 calves was produced by 152 cows. The greatest number of cows produced 12 calves; however, 76 cows were allowed opportunity to bear only 12 calves. If these had had opportunity to bear 13 calves, as were the other 76 cows, 13 would likely be the most common number.

As dictated by the criterion "failure to deliver a calf during two successive years," 83 of 152 cows were infertile at the end of the study. A frequency distribution of these cows with respect to age at infertility is shown in Fig. 2. Two years is the initial point since within the limits of our definition of infertility a cow would be at least that old before she could be declared infertile. The greatest number became infertile at 11 years, but 13 was the age at which 55 percent of the herd became infertile. In Fig. 3, the data from the frequency distribution were plotted with the logit (Bliss, 1970) of infertility as a function of age. Transformation of cumulative incidence of infertility to logits resulted in a straight line relationship (r^2 = 0.99) between infertility and age described by the regression equation,

$$Y = \ln \frac{P}{1-P} = a + b \text{ age},$$

where a = −3.6903 and b = 0.2995. In light of the apparent "goodness of fit" of data to the model, it should not be unreasonable to calculate from the model the total reproductive capacity of the Hereford female. Since only one-half of the cows were observed through their 15th year, it is necessary to estimate the reproductive performance of the residual at the 15th year and the total incidence of infertility at the 16th and all succeeding years. The logit curve is asymptotic; therefore, to make the

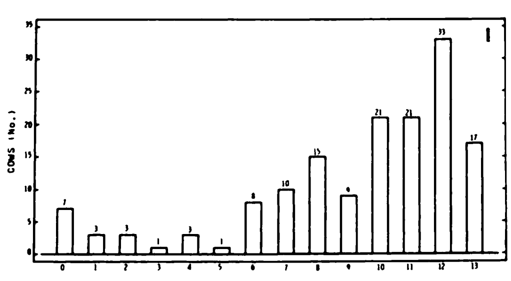

FIG. 1. Distribution of 152 grade Herefords according to their reproductive performance to 14 to 15 years of age. Number above bar is number of cows.

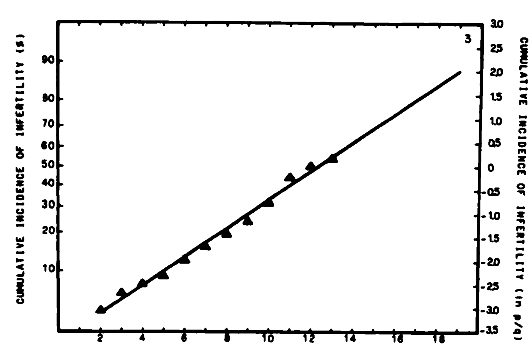

FIG. 3. Cumulative incidence of infertility as a function of age. A regression of logit of incidence of infertility on age yielded the equation, Y = ln (P/1-P) = a + b age, where a = −3.6903 and b = 0.2995.

TABLE 1. Relationship between ovarian follicular endowment and the reproductive state (fertile vs. infertile)[a] of the aged cow (14 to 15 years).

Reproductive state	Cows (no.)	Follicular class[b]				
				Vesicular		
		Primordial (no. X 10^3)	Growing (no.)	Normal (no.)	Atretic (no.)	Total
Fertile	69	25 ± 3[c]	268 ± 19	41 ± 3	26 ± 2	67 ± 5
Infertile	78	23 ± 3	204 ± 15[d]	40 ± 3	27 ± 2	67 ± 4

[a]A cow was judged infertile if she failed to deliver a calf during two successive years.

[b]Primordial – oocyte surrounded by a single layer of follicular cells; Growing – from an oocyte surrounded by two or more complete layers of follicular cells to a follicle with an incomplete vesicle; Atretic – from widespread necrosis among the follicular cells to first evidence of fibroblastic invasion of the vesicle.

[c]Mean ± standard error.

[d]Significantly different from fertile ($P<0.01$).

estimate conservative, 90 percent infertility was selected as the terminal point. And, as shown in Fig. 3, this figure corresponds approximately to an age of 19 years. Reports of reproduction in cattle 19 years or greater are extremely limited. Smith and Robinson (1931) found, however, as a result of analysis of Herd Book records, that the oldest productive animal was 21 and only five were productive beyond the 17th year. Also, of 16 Herefords allowed to live a lifetime at our laboratory the oldest died when 20 years of age. Therefore, assuming the 14-year-old cows ($N = 76$) that had opportunity to bear only 12 calves would bear 13 calves in the same proportion as the 15-year-old cows (17 calves from 76 cows) and that the incidence of infertility at 16 would be 75 percent, 80 percent at 17, 85 percent at 18 and 88 percent at 19 (Fig. 3), an additional 66 calves would be added to the observed total of 1406. Consequently, the calculated mean reproductive capacity of the Hereford cow becomes 10.4 calves.

Ovarian follicular populations of fertile and infertile cows are recorded in Table 1. Fertile ovaries nonsignificantly ($P>0.25$) exceeded infertile ovaries in number of primordial follicles by 8 percent, but in number of growing follicles fertile exceeded infertile by 24 percent—a difference that was significant at the 1 percent level of probability. No intergroup difference occurred, however, in either the number or quality (normal or atretic) of vesicular follicles. It appears, therefore, that in ovaries from infertile cows there is a deficiency in the transfer of follicles from the primordial to the growing state, but a deficiency was not evident in the transfer of follicles from the growing to vesicular state.

A frequency distribution of cows based on their germ-cell complement is displayed in Fig. 4. The median fell between 11 and 20 thousand and the mean was 24 ± 3 thousand—an estimate not significantly different from that previously reported by Erickson (1966) for similarly aged animals. Fourteen of the 16 animals with less than 1,000 germ cells were infertile, but the ratio of fertile-to-infertile within the other classes did not depart significantly from the ratio seen in the population as a whole (69 fertile to 78 infertile). From this we conclude that only 20 percent of the infertility could be

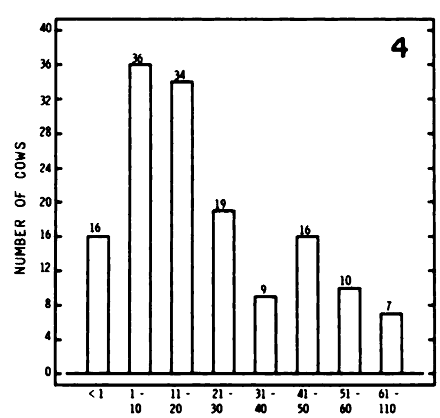

FIG. 4. Frequency distribution of 147 aged cows (14 to 15 years) relative to germ-cell endowment. Number above bar is number of cows.

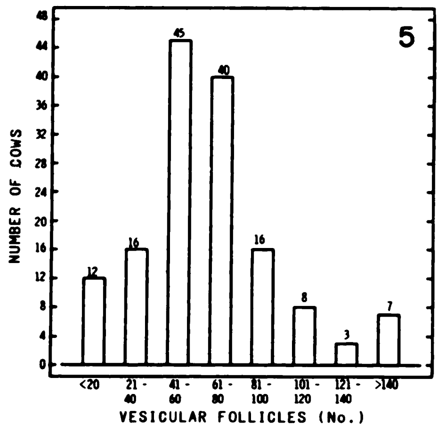

FIG. 5. Frequency distribution of 147 aged cows relative to total vesicular-follicle endowment. Number above bar is number of cows.

ascribed to a lack of germ cells (16 cows with <1,000 germ cells divided by total number of infertile cows—78). When the cows were distributed according to their total vesicular follicular endowment, the pattern shown in Fig. 5 emerged. The median was between 41 and 60 and the mean was 67 ± 5. In no group did the ratio of fertile-to-infertile depart significantly from expectation. Thus, even in the group marked by less than 20 follicles only slightly less than one-half of the cows were infertile. Consequently, number of vesicular follicles appears to be a poor indicator of reproductive success.

To test the degree of interdependence between the three follicular classes, correlation coefficients were calculated without regard to reproductive status. The positive coefficient for primordial versus growing was 0.39; primordial versus total vesicular, 0.50; growing versus total vesicular, 0.40; and normal versus atretic vesicular follicles, 0.74. All coefficients were significant ($P<0.01$).

Incidence and type of abnormality found in the ovaries of the infertile animals are recorded in Table 2. Of the six kinds of abnormalities observed, the cystic corpus luteum accounted for 56 percent of the total. Anovulatory ovaries were of next importance (19 percent of total) and adhesions were of least importance (3 percent of total). Ovaries in 65 of the 78 infertile cows were abnormal; hence, ovarian pathology accounted for 83 percent of the infertility. In no case was a cystic corpus luteum found in ovaries from fertile cows; and within the experience of the authors, a cystic corpus luteum has not been found in association with pregnancy—a finding supported by the work of McEntee (1958).

DISCUSSION

Previous estimates of the mean reproduction capacity of the bovine have not excluded mortality as a cause of infertility. Consequently the means given for the Harianna (4.5) and Tharparker (5.8) breeds by Kohli and Suri (1957) were considerably less than those observed (9.3) and calculated (10.4) by us. Mortality was a matter of considerable consequence in the Harianna, since 91 percent of a herd of 1917 cows succumbed to a variety of diseases by the 14th year, and the upper age limit of the

TABLE 2. Incidence and type of abnormality in ovaries of the infertile bovine[a]. (Cows were 14 to 15 years of age when slaughtered.)

Abnormality (no. of cows affected)							Total cows affected	
Adhesions[b]	Cystic[c] follicles	Luteinized[d] follicles	Cystic[e] corpus luteum	Anovulatory[f]	Afollicular	Total	No.	%
2	7	5	42	14	5	75	65	83

[a]Ovaries of the fertile cows were apparently free of abnormalities.

[b]Ovaries adhered to surrounding tissues.

[c]Follicles devoid of both a granulosal layer and an intense affinity for nuclear stain in the theca interna.

[d]Follicles devoid of a granulosal layer but marked by a luteinized theca interna.

[e]Corpus luteum with a fluid-filled center.

[f]Ovaries devoid of a corpus luteum.

breed was reported to be 18 years (Kohli and Suri, 1957). In our herd of 219 Herefords only 31 percent had died by the 14th year. Cows died at an average rate of 2 percent per year, and there was no evidence of an increase in mortality rate with increase in age.

The mean life expectancy of the bovine has been estimated to be 19 years (Asdell, 1946), and the most advanced age reported has been 30 years (Hammond and Marshall, 1952). Given an average age at infertility of 13 years (Fig. 2) and an average age at death of 19 years, it is evident that the average bovine female, like other mammals (Talbert, 1968), will live beyond her reproductive years. Relative to mean reproductive capacity within another monotocous species, it is interesting to note that in a segment of the human population where birth control measured were proscribed, large families encouraged and health care superior, average number of children per mother was 10 (Eaton and Mayer, 1953).

Age-related infertility has been attributed to a number of causes (see Talbert, 1968, for review) and one of the first explored was the germ cell (Ingram et al., 1958; Jones and Krohn, 1961). Since in all mammalian species studied the germ-cell population diminishes with age and reaches zero or near-zero levels in females of advanced age (Talbert, 1968), germ-cell depletion would appear to be the principal cause of infertility. However, it has been observed in a variety of species, including the bovine (Table 1), that infertility precedes germ-cell depletion, but even with an apparently adequate germ-cell supply and a normal rate of ovulation, infertility in the hamster (Thorneycroft and Soderwall, 1969) and mouse (Harmon and Talbert, 1970) is preceded by a reduction in litter size.

Germ-cell quality has been tested through experiments involving the transfer of premorula embryos from aged donors to young hosts and vice versa. Results of studies in the hamster (Blaha, 1964) showed that reduced germ-cell quality was a significant cause of reduced litter size, but a defective uterine environment was also implicated. In another test of germ-cell quality in the golden hamster, Parkening and Soderwall (1975) found that approximately 40 percent of the oocytes ovulated by senescent females were nonviable at the time of implantation; they therefore concluded that preimplantation loss was the most important factor in declining litter size. Lowered germ-cell quality was apparently a factor of minor importance in the mouse, but again incomplete responsiveness of the uterus was cited as a cause of reduction in litter size (Talbert and Krohn, 1966). In support of uterine refractoriness as a cause of lowered fertility, Finn (1966) found that the decidual cell reaction in uteri of aged mice was considerably reduced.

Attempts to explain the refractoriness of the uterus have led to studies of the corpus luteum. In senescent hamsters Thorneycroft and Soderwall (1969b) found that corpora lutea were reduced in size and number; and, in the mouse, corpora lutea of aged females commonly degenerated (Harmon and Talbert, 1970). Consequently, the researchers of both species concluded that the uterus was not adequately supported by the corpus luteum. Defective corpora lutea have also been implicated in infertility problems in the human female (Collett et al., 1954; Novak, 1970); and, as was seen in Table 2, a defective corpus luteum is probably the most common cause of infertility in the bovine.

The abnormal corpus luteum and possibly the anovulatory ovary in the aged female could be due to one or all of the following: 1) inability of follicular cells (granulosal and theca interna) to fully respond to hormonal stimuli; 2) change in the quantity and/or quality of hormone secretion; 3) reduced stimulus for hormone secretion. To date, the importance of the first item has not been tested, and this is essentially the case with the second. It is known, however, that the hormonal status of the human female changes radically at menopause (see Finch, 1965, for review) as does the hormonal status of the postreproductive female rat (Clemens and Meites, 1971). With respect to the third item, the work of Peng and Peng (1973) suggests that the estradiol-binding capacity of the hypothalamus in aged female rats is diminished, and Finch (1973) has shown that the rate of catecholamine synthesis in the hypothalamus is reduced in the aged mouse; but whether or not changes in the hormonal milieu precede loss of reproductive efficiency is not known. Thus, definitive explanations for the limitation on or loss of fecundity await the results of studies that are far more complete than those available today.

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