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DEVELOPMENT AND RADIO-RESPONSE OF THE PRENATAL BOVINE OVARY*

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Summary. Qualitative observations of forty-nine bovine foetuses taken at varying stages of gestation revealed the following: (1) the germinal ridge is established at the 32nd day of gestation; (2) due to the formation of the tunica albuginea of the primitive testis, foetal sex is distinguishable by Day 39; (3) meiosis begins in ovaries at 75 to 80 days *post coitum*; (4) oogonial mitosis is discontinued by 150 to 170 days; (5) the majority of the oocytes have attained their stage of rest (pachytene) and are enveloped in primordial follicles by Day 170; and (6) vesicular follicles appear in ovaries at Day 250 of gestation.

Total germ-cell numbers, based on volumetric determinations or direct counts, increased from a low of approximately 16,000 at Day 50 to a high of 2,700,000 at Day 110. From Day 110 numbers declined abruptly to 107,000 at Day 170 and the ovaries of four foetuses at 270 days of gestation (average length of gestation, 283 days) contained an average of 68,000 ± 21,000 germ cells.

Seventy prenatally irradiated (400 R, whole-body to dam) and twenty-five non-irradiated heifers were slaughtered at either 9 or 23 months of age. Microscopic examinations of their ovaries revealed that irradiation was apparently without effect during the interval of 17 to 59 days of gestation, and of questionable effect during the interval of 59 to 119 days of gestation. Irradiation administered during the 119- to 154-day interval, however, diminished germ-cell numbers by an average of 68%. From Day 154 to term, irradiation was again without effect. The mitotically active oogonium was probably the cell affected during the interval of 59 to 119 days of gestation and the drastic germinal decline during the 119- to 154-day interval was believed to be related to the progression of oocytes from zygotene to pachytene, a transition during which the oocyte under normal conditions is highly susceptible to death.

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INTRODUCTION

Little is known regarding the prenatal development of the bovine ovary, and data concerned with the effects of ionizing radiation on the foetal ovary of this species are limited to a fertility test of a single-conception duration conducted by Parish, Murphree & Hupp (1962). No difference was noted between control and irradiated heifers.

Papers by Henricson & Rajakoski (1959) and Mauléon (1961) have contributed to an understanding of some qualitative aspects of prenatal ovarian development, but no attempt has been made to interpret quantitatively the succession of events associated with the evolution of the prenatal bovine germ cell.

The present study was undertaken primarily to determine the effects of ionizing radiation on the bovine germ cell at varying points in its prenatal life cycle. A secondary objective was to determine through quantitative and qualitative observations of the non-irradiated foetal ovary if changes in the radio-sensitivity of germ cells could be correlated with stages of major change in morphogenesis.

MATERIALS AND METHODS

PRENATAL OVARIAN DEVELOPMENT

Animals

Gonads or ovaries from forty-nine foetuses varying in age from 32 to 270 days of gestation were obtained at slaughter. All foetuses were the products of controlled matings of animals within the laboratory's herd of grade Herefords. The day of mating was considered 'Day 1' of gestation.

Histology

All tissues were fixed in Lavdowsky's mixture (Guyer, 1949), sectioned serially at 7 μ and stained with Iron Haematoxylin and Orange G.

For quantitative assessments of total numbers of germ cells in ovaries aged up to 150 days of gestation, the combined techniques of Chalkley (1943) and Dornfeld, Slater & Scheffé (1942), as employed by Beaumont & Mandl (1962), were used. Through the method of Chalkley, with the exception of Day 50 (one animal), 600 cells were scored as to germinal or somatic character in the largest sagittal section of one ovary of each of three animals at fourteen selected stages of gestation (Table 1). Likewise, the volume of at least one ovary of each of three foetuses at points up to Day 170 was calculated after the method of Dornfeld *et al.* (1942).

From measurements of at least twenty cells in each category, the average volumes for oogonia and oocytes at all stages of meiosis were 1055 and 1901 μ^3, respectively. As the ratio of oogonia to oocytes changes with advances in ovarian development, the mean cellular volume was weighted accordingly. When the percentage of germinal tissue, total ovarian volume and the mean volume for germ cells are known, the total number of germ cells can be computed (*see* Beaumont & Mandl, 1962, for details). Numbers of mitotic figures, necrotic germ cells, oogonia and oocytes in leptotene, zygotene or pachytene

of meiotic prophase were determined by direct counts of the cells in two of the largest sagittal sections in one ovary of at least two foetuses at each stage of gestation (Table 1).

Beyond Day 150, all determinations were made from direct counts of every 10th section of serially sectioned ovaries (at least one ovary from each of three foetuses at each stage of gestation considered). To avoid spuriously high oocyte counts, only those oocytes appearing in cross section with the major portion of their chromatin mass intact were counted.

RADIO-RESPONSE OF THE PRENATAL BOVINE OVARY

Animals and irradiation

A total of ninety-five Hereford females were used. With the balance of this number serving as controls, seventy gravid cows were exposed to a whole-body dose of 400 R (roentgens) of γ-radiation (cobalt-60, measured in air at skin level) at an approximate rate of 0·7 R/min. Distribution of animals with respect to treatment and stage of pregnancy irradiated are recorded in Table 2, and a description of the radiation facility appears in an article by Wilding, Simons & Rust (1952). Based on a study of Brown, Thomas, Jones, Cross & Sasmore (1961), who employed this same facility, it is estimated that the foetuses irradiated in this study received 35 to 50 % of the air dose. The prenatally irradiated heifers were slaughtered when aged either 9 or 23 months *post partum*.

Histology

Ovaries were bisected at their point of greatest circumference, fixed in Bouin's alcoholic fluid and sectioned at a thickness of 7 μ. Stains were as listed for the non-irradiated prenatal ovaries.

Primordial follicles (oocytes encompassed by no or one layer of follicle cells), growing follicles (oocytes with two or more layers of follicle cells, but without fully-formed vesicles) and vesicular follicles were counted in five sections taken at 100 μ intervals from each 'cut' ovarian surface (ten sections/ovary or twenty sections/animal). To avoid duplication in counts of vesicular follicles, the highest count of the ten sections from each ovary was taken as representative of the vesicular follicle population of that ovary.

RESULTS

DEVELOPMENT OF THE PRENATAL OVARY

The germinal ridge or primitive gonad was not observed in serial sections of a specimen aged 30 days *post coitum*, but it was present in rudimentary form in all three specimens aged 32 days *post coitum*. Although not seen earlier, germ cells were distinguishable in gonads aged 35 to 36 days of gestation (three foetuses), and with the formation of the tunica albuginea in the primitive testis, sex was determinable in a specimen of 39 days gestation.

The foetal bovine ovary from Day 40 to Day 70 is apparently without internal organization, but somatic and germinal elements are distinguishable

microscopically (Pl. 1, Fig. 1). Meiosis is initiated at Day 75 to Day 80 and, with this, ovigerous cords are formed, and the ovary becomes divided into cortical and medullary parts (Pl. 1, Fig. 2). Oogonial mitosis is discontinued at or near 150 days of gestation, the ovigerous cords are disrupted, and by Day 170 the ovary is characterized grossly by a narrow cortical band of germinative tissue and a prominent medulla (Pl. 1, Fig. 3). With the disruption of the ovigerous cords, the vast majority of surviving oocytes acquire a single layer of flattened follicle cells and thus form primordial follicles. Vesicular follicles were first seen in ovaries aged 250 days *post coitum* and by Day 270 the ovary in one instance became a mass of vesicular follicles (Pl. 1, Fig. 4).

Quantitative data concerning bovine prenatal ovarian development are

TABLE 1

DEVELOPMENT OF PRENATAL BOVINE OVARY

Stage of gestation (days)	*Combined* ovarian weight (mg)*	*Combined* ovarian volume (mm^3)*	*No.† mitotic figs.*	*Total‡ germ cells*	*Germ§ cells necrotic (%)*	*Oogonia§ (%)*	*Oocytes§*		
							Leptotene (%)	*Zygotene (%)*	*Pachytene (%)*
50	7	0·84	13	15,924	0	100	—	—	—
60	28 ± 3	1·70 ± 0·22	304	322,275	0	100	—	—	—
70	36 ± 4	5·66 ± 1·31	142	1,073,000	0	100	—	—	—
80	46 ± 9	9·48 ± 0·94	214	2,183,000	23	38	21	41	0
90	52 ± 5	9·37 ± 1·67	182	2,043,000	28	36	17	45	2
110	101 ± 17	14·18 ± 3·15	223	2,739,000	25	36	21	39	4
130	132 ± 10	23·73 ± 4·26	69	2,448,000	28	30	9	36	25
150	138 ± 12	27·89 ± 6·78	3	1,528,000	34	9	4	24	63
170	164 ± 22	—	0	107,600	28	3	2	6	89
190	264 ± 14	—	0	81,060	18	3	0	0	97
210	423 ± 55	—	0	81,520	6	12	0	0	88
230	482 ± 34	—	0	116,200	11	7	0	0	93
250	645 ± 60	—	0	51,513	6	2	0	0	98
270	1319 ± 601	—	0	68,150	0·1	2	0	0	98

* With the exception of Day 50, individual values represent an average of at least three animals.
† Average number observed in two largest cross-sections of a single ovary from two animals.
‡ Numbers were determined volumetrically through Day 150 and by direct perusal of serial sections thereafter.
§ Percentages were determined through direct cell counts in two largest cross-sections of a single ovary from two animals.

recorded in Table 1. Ovarian weight rises abruptly between Days 50 and 60 in conjunction with a pronounced rise in germ-cell numbers and again at Day 110 to coincide with the establishment of the peak number of germ cells. The great increase and great variability in ovarian weights seen at Day 270 were both due to the development of vesicular follicles.

Changes in numbers of mitotic figures, as would be expected, paralleled closely the rise and fall of total germ-cell numbers. Day 60 apparently marks the beginning of intense oogonial mitotic activity. At or near Day 150 oogonial mitosis is discontinued and, with this, the number of germ cells available to the bovine female is fixed.

The changes in total germ-cell numbers can be described as nothing less than spectacular. In the course of approximately 60 days (Day 50 to Day 110),

PLATE 1

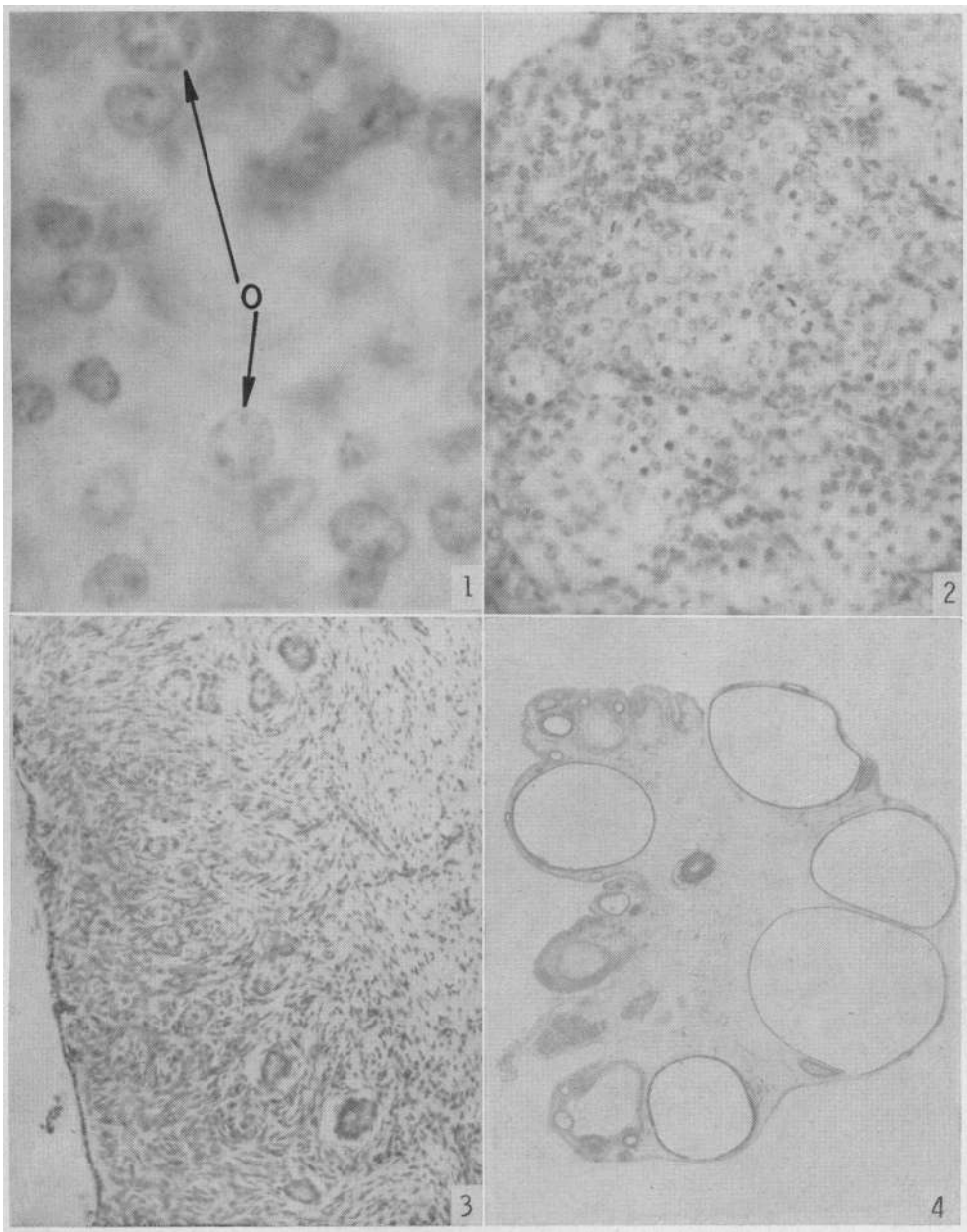

FIG. 1. Prenatal bovine ovary, Day 50. Note mixture of germinal (o) and somatic elements. × 1064.

FIG. 2. Prenatal bovine ovary, Day 80. Ovary now divided into cortical and medullary parts. Note ovigerous cords and attendant oocytes in cortical area. × 203.

FIG. 3. Prenatal bovine ovary, Day 170. Note presence of primordial follicles and absence of ovigerous cords. × 102.

FIG. 4. Prenatal bovine ovary, Day 270. Note growing and vesicular follicles. × 4·5.

(*Facing p.* 100)

PLATE 2

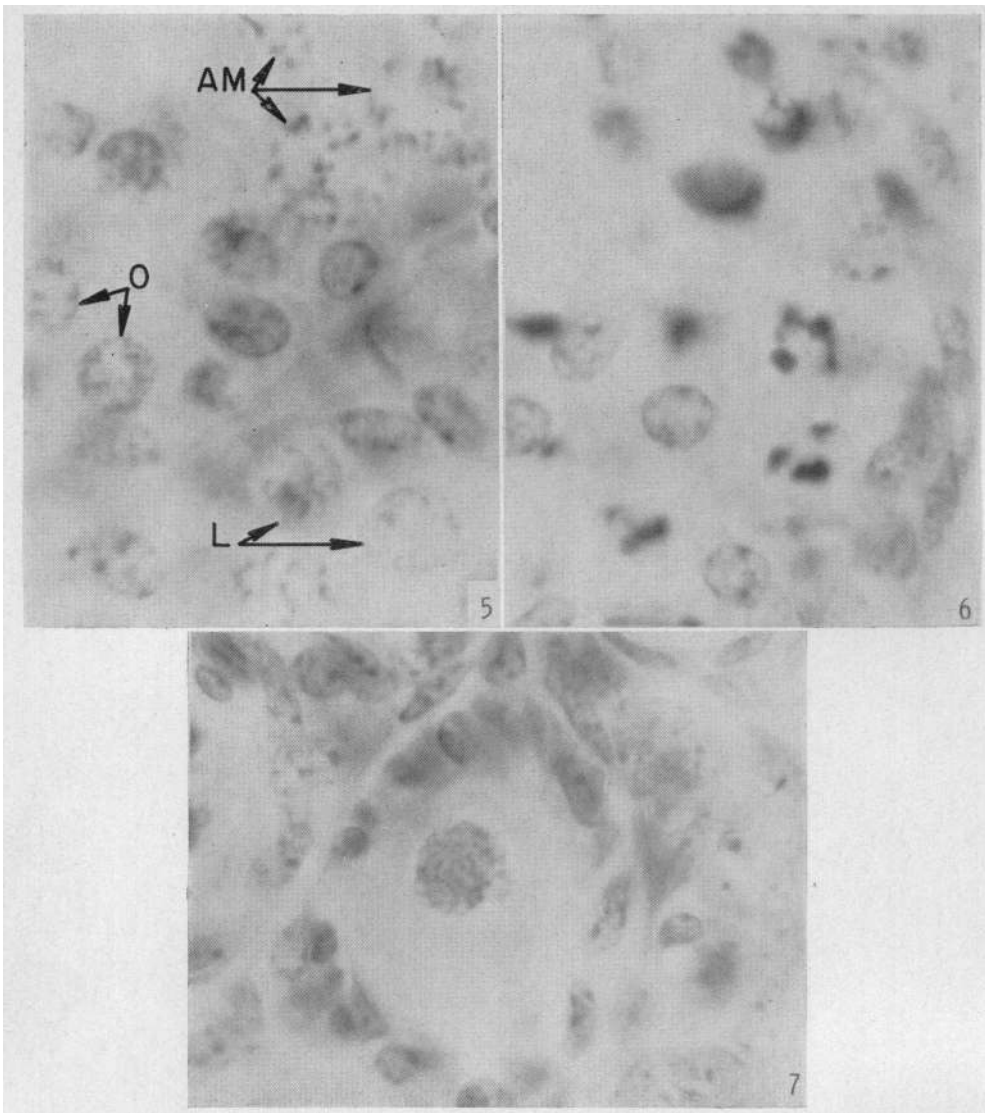

FIG. 5. Prenatal bovine ovary, Day 90. Aberrant oogonial mitotic figures or degenerating premeiotic oocytes (AM). Normal oogonia (O). Oocytes in leptotene (L). × 1148.

FIG. 6. Prenatal bovine ovary, Day 120. Areas of intense pigmentation mark degenerating oocytes ('Z' cells). Cells in middle and lower segments of the figure represent more advanced stages of degeneration. × 1148.

FIG. 7. Bovine oocyte at stage of rest (pachytene). × 1148.

germ-cell numbers increase from 16,000 to 2,700,000, and in a period of 40 days (Day 130 to Day 170) the germ-cell population is diminished from 2,500,000 to 108,000. This decline is linked with the cessation of oogonial mitosis and a high rate of cellular necrosis. Direct germ-cell counts that were undertaken with foetal ovaries aged 170 to 270 days revealed a range of individual differences in total numbers of germ cells that varied from 18,000 to 200,000. Standard errors for the five age groups involved (three animals/group) varied from 19,600 to 42,300, thus explaining the inordinately high value seen at Day 230.

Beaumont & Mandl (1962) described four 'waves' of degeneration of germ cells in the prenatal female rat. The first of these, although limited in extent, affects oogonia prior to the onset of meiosis; the second is associated with the beginning of meiosis and affects the dividing oogonium; the third affects oocytes principally at the pachytene stage of first meiosis (referred to as 'Z' cells), and the fourth affects the oocyte at diplotene. As recorded in Table 1, no necrosis was observed among the oogonia of the bovine prior to the initiation of meiosis (Day 80). With the beginning of meiosis, however, great numbers of aberrant oogonial mitotic figures or degenerating premeiotic oocytes appeared. These figures, similar to those described for the rat by Beaumont & Mandl (1962) and pictured in Pl. 2, Fig. 5 for the bovine, were characterized by the presence of ranks of spherically-shaped clumps of chromatin. This form of necrosis predominated until Day 130 of gestation, when a pronounced drop in numbers of normal germinal mitotic figures and a major shift of oocytes from earlier stages of meiosis to the pachytene stage brought on a preponderance of the third form of germinal degeneration or the 'Z' cell. From Day 150 to Day 270 the 'Z' cell was the only form of germinal necrosis observed. A 'Z' cell (Pl. 2, Fig. 6) is distinguished by condensation of chromatin threads and cytoplasmic acidophilia. Since according to our observations the bovine oocyte 'rests' at pachytene (Pl. 2, Fig. 7), the fourth 'wave' of germinal degeneration described by Beaumont & Mandl was not observed. The prenatal bovine germ cell thus passes through two developmental phases in which the prospects for its further development are quite tenuous. The first phase is apparently the oogonial mitotic division that gives rise to the oocyte, and the second phase occurs either as the oocyte enters or is in pachytene of meiotic prophase.

RADIO-RESPONSE OF THE PRENATAL BOVINE OVARY

Based on data recorded in Table 2, two periods in gestation emerge as probable times of germ-cell radio-sensitivity. If the reductions in germ-cell numbers noted at Days 59 to 62 and Days 90 to 100 are real, the first of these periods would extend from Day 59 to approximately Day 119. The period 90 to 100 days *post coitum* possesses no qualities that could distinguish it from immediately preceding and succeeding periods, but the 59- to 62-day interval is distinguished from its preceding period by the initiation of a high oogonial mitotic rate that extends to a point between Day 110 and Day 130 *post coitum* (Table 1). The assumption of an irradiation effect is valid here only if it is reasoned that germ-

cells were destroyed to the same extent in the ovaries of all animals in each of the four groups represented. Failure to note an effect in the ovaries of animals irradiated at 69 to 80 and 109 to 111 days of gestation could be ascribed to their inherently high germ-cell numbers and an irradiation effect actually of small proportions that was magnified by the inherently low germ-cell numbers possessed by the animals irradiated at 90 to 100 days of gestation.

Distinctive from the first period was the extensive germ-cell death effected by γ-radiation during the interval of 119 to 154 days of gestation. This period (Table 1) in the morphogenesis of the germ-cell population is distinguished by a cessation of oogonial mitosis, a high rate of germ-cell necrosis, and a progression of the oocyte population to the pachytene condition.

Counts of growing and vesicular follicles would support a contention that

TABLE 2

EFFECT OF γ-RADIATION (400 R TO DAM) ON THE GERM CELLS OF THE PRENATAL BOVINE OVARY

Age at irradiation (*days* p.c.)	*Age at slaughter* (*months* p.p.)	*No. animals*	*No. primordial* follicles*	*No. growing follicles*	*No. vesicular follicles*	*Primordial follicles* (% *of cont.*)
Control	9	5	1925 ± 487†	22 ± 3	25 ± 3	—
Control	23	20	1337 ± 292	16 ± 2	24 ± 3	—
17 to 23	23	18	1828 ± 246	20 ± 3	26 ± 1	137
27 to 34	9	7	2168 ± 373	19 ± 2	21 ± 3	113
40 to 54	23	4	1561 ± 417	20 ± 2	25 ± 1	117
59 to 62	9	2	1665 ± 25	18 ± 6	21 ± 6	86
69 to 80	9	4	2043 ± 457	19 ± 3	20 ± 2	106
90 to 100	9	4	1213 ± 560	15 ± 3	23 ± 3	63
109 to 111	9	3	2037 ± 670	24 ± 3	41 ± 12	106
119 to 132	9	5	729 ± 308	14 ± 1	13 ± 2	38
148 to 154	9	7	862 ± 267	13 ± 1	20 ± 3	45
160 to 180	23	8	1767 ± 348	18 ± 2	26 ± 5	132
210 to 234	9	4	1577 ± 755	17 ± 5	25 ± 7	82
260 to 280	9	4	2378 ± 482	27 ± 10	35 ± 4	124

* Primordial (oocytes encompassed by 0 to 1 layer of follicle cells), growing (oocytes with two or more layers of follicle cells, but without fully-formed vesicles), and vesicular follicles were counted in five slides (100 μ intervals) prepared from each of the 'cut' surfaces of ovaries bisected at their point of greatest circumference (ten slides/ovary or twenty slides/animal).

† Mean ± standard error.

the follicle-forming ability of oocytes that survive the quantity and quality of radiation delivered here is unimpaired. Normal oestrous behaviour was observed among those animals reared to 23 months of age, and all carried apparently normal foetuses at slaughter.

The extreme variability in oocyte numbers between individuals was an unexpected find of this study. If animals irradiated at days of gestation other than 90 to 100 and 119 to 154 are added to the controls, a population of seventy-four bovine females could be distributed with respect to germ-cell numbers, as follows: number of animals with 300 to 800 oocytes per twenty ovarian cross-sections (ten sections/ovary at 100 μ intervals) = 17; number with 1000 to 2500 oocytes = 46; and number with 3000 to 5000 oocytes = 11. Although oocyte numbers in ovaries of controls aged 23 months are somewhat

lower than in those aged 9 months (Table 2), if animals irradiated at 17 to 23 days of gestation are taken as representative of their particular age group, it could be concluded that very few primordial follicles undergo atresia during the time between 9 and 23 months.

To test how reliably our sampling technique reflected the total population of oocytes, the ovaries of six animals (three from the highest of the controls and three from the group irradiated at 119 to 154 days of gestation) were serially sectioned. Oocytes in every 20th section or less (where high variability in successive counts warranted) were counted, and average population differences between the sample and total counts were compared. From the sample of twenty cross-sections, the average numbers of oocytes in the low and high populations were 1758 ± 223 and $22{,}243 \pm 1830$, respectively. Following counts of serial sections, the respective totals were $12{,}553 \pm 2830$ and $149{,}797 \pm 6860$. In the first instance, the high population exceeded the low by 80% and in the latter instance, the high exceeded the low by 84%. Thus, a high degree of confidence in the sampling technique is indicated.

The question of the relative distribution of the germ-cell population between right and left ovaries was considered through oocyte counts in serial sections of eight additional non-irradiated animals. Of the eleven ovarian pairs examined, the number of oocytes in each ovary differed by less than 10% in eight cases. In two subjects the right exceeded the left by 36% and 39%, respectively, and in one case, the left exceeded the right by 15%. From the standpoint of probabilities then, enumeration of germ cells in a single ovary is likely to provide a satisfactory estimate of the population in the opposite ovary.

DISCUSSION

PRENATAL OVARIAN DEVELOPMENT

This study substantiates the observations of Henricson & Rajakoski (1959) and Mauléon (1961) regarding the stage in gestation at which meiosis begins, primordial follicles appear and vesicular follicles are formed. Time of gonadal differentiation is closely allied with that reported by Krehbiel (1963), but the literature is believed to contain no within-species parallel to other qualitative and all-quantitative information reported herein. Several developmental stages in the prenatal evolution of the bovine ovary and its germ cells, however, mimic closely in time of occurrence those described for man. Establishment of the germinal ridge, definitive ovary, first-primordial follicles, majority of oocytes in primordial follicles and vesicular follicles is completed at approximately Days 32, 40, 130, 170 and 250 of gestation, respectively, in both the human (Potter, 1962; Van Wagenen & Simpson, 1965) and bovine (present study). In addition, Baker (1963) has reported that: (1) meiosis is initiated in the human ovary at 70 to 80 days of gestation (75 to 80, bovine), (2) the maximum number of germ cells is attained at Day 150 (110 to 130, bovine), (3) a plurality of pachytene oocytes occurs at Day 120 to Day 150 (130 to 150 bovine), (4) the highest level of germinal necrosis occurs at 120 to 150 days of gestation (120 to 150, bovine), and (5) oogonial mitosis is discontinued at

approximately Day 210 (150 to 170, bovine). A point of major divergence in germinal evolution between the two species is that the oocyte of the bovine rests at pachytene of the meiotic prophase (Henricson & Rajakowski, 1959; Erickson, 1965 and present study), and the human oocyte rests at diplotene (Baker, 1963).

RADIO-RESPONSE OF THE PRENATAL BOVINE OVARY

Of the limited number of studies designed to test the ability of the prenatal mammalian female germ cell to survive an exposure to ionizing radiation, the works of Beaumont (1961, 1962) are probably the most thorough. She interpreted the response of the prenatal rat through counts of oocytes surviving to 25 days *post partum* and found that 100 R of X-rays inflicted negligible damage to the germ-cell population prior to Day 15 of gestation, but radiation administered at Day 15 reduced oocyte numbers to 15% of control values. Increased sensitivity at Day 15 was thought to be due to the high rate of oogonial–mitotic activity noted in ovaries of similarly aged controls. Following irradiation at Day 17 *post coitum*, when the majority of the oocytes, according to her observations, enter leptotene, numbers of surviving oocytes rose to approximately 50% of control, and at Day 19, with a preponderance of oocytes in zygotene, 90% of the oocytes survived 100 R of X-radiation. Approximately 60% of the oocytes survived 100 R on the day of birth, when the pachytene chromosomes predominate, but virtually all germ cells are destroyed when the same dose is delivered on Day 5 *post partum*, as the bulk of the rats' oocytes attain their stage of rest (Beaumont, 1962).

The response of the prenatal bovine germ cell to ionizing radiation parallels to some extent that described for the rat. Prior to Day 60 (Table 2) 400 R of γ-radiation (whole-body to dam) caused no observable germ-cell death, but at Day 60 and the beginning of a high oogonial mitotic rate, a 14% drop (a decline of doubtful significance) in total germ cells occurred. If the mitotically active oogonium was affected here, a similar effect should prevail through at least Day 120 of gestation (Table 1). The response elicited in germ cells irradiated during the 80- to 120-day interval could support Beaumont's observation indicating a considerable radio-resistance for the leptotene oocyte, but the response seen at 120 to 150 days of gestation in the bovine is apparently without parallel in the rat. Here, the necrotizing effects of radiation were most probably acting on a transitional oocyte (zygotene to pachytene). This contention is supported by the fact that the zygotene oocyte predominates during the interval preceding the stage of presumed sensitivity and the pachytene oocyte is dominant throughout the succeeding gestational stages. The respective radiation responses of resting bovine and rat oocytes are widely divergent. Whereas 100 R of ionizing radiation will destroy the rat oocyte in its stage of rest or dictyate (Beaumont, 1962), the resting bovine oocyte (pachytene) will tolerate an acute localized exposure of γ-radiation in excess of 600 R (Erickson, 1965).

Germ-cell counts can obviously provide information as to the existence of an irradiation effect, but their ultimate significance relative to reproductive

capacity awaits further study. In the bovine, the relationship between reproductive capacity (female's lifetime reproductive performance) and germ-cell numbers assumes considerable importance in the light of the irradiation effect in the prenatal animal and the wide difference between individuals in a presumed normal population. The individual differences and distribution of this population could prompt the following questions: (1) Is the reproductive capacity of those animals with low numbers of germ cells equal to those animals which occupy intermediate and high positions or are those animals with high numbers superior to those in lesser positions? (2) To what extent are high germ-cell numbers heritable? (3) What is the minimum number of germ cells necessary for normal ovarian function? (4) What is the relationship between germinal quantity and certain somatic qualities?

Studies relevant to these questions are in progress.

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