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REPEATABILITY OF NUMBERS OF ANTRAL FOLLICLES IN FOLLICULAR WAVES IN DAIRY CATTLE AND ASSOCIATION WITH SECRETION OF FSH, ESTRADIOL AND INHIBIN-A

presented by

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Major Professor's Signature

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By

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ABSTRACT

REPEATABILITY OF NUMBERS OF ANTRAL FOLLICLES IN FOLLICULAR WAVES IN DAIRY CATTLE AND ASSOCIATION WITH SECRETION OF FSH, ESTRADIOL AND INHIBIN-A

By

David S. Burns

The objective of this study is to examine the variation in antral follicle numbers that develop during follicular waves in cattle, and to determine if the variation in numbers of follicles per wave is associated with serum FSH, estradiol and inhibin-A concentrations. Cattle were subjected to twice daily ultrasound analysis to count number of antral follicles during different follicular waves in estrous cycles. The results demonstrated that maximal numbers of antral follicles in different follicular waves were highly variable amongst cattle, but remarkably highly repeatable (0.95) within individuals. In another experiment, cows that consistently had very high or low numbers of antral follicles per wave were identified and blood samples were taken. Serum was subjected to FSH, estradiol and inhibin-A immunoassays. The results demonstrated that serum FSH concentrations were 50% lower in cows with very high numbers of antral follicles per wave *vs* cows with low numbers. However, estradiol and inhibin-A were similar between the two groups of cows. These results led us to conclude that: a) a chronic compensatory mechanism must exist in ovaries to maintain growth of a constant number of antral follicles in follicular waves, and b) the variability in numbers of follicles per wave is probably not explained by the interaction of FSH, with estradiol and inhibin-A concentrations.

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LIST OF ABBREVIATIONS

FSH	follicle stimulating hormone
LH	luteinizing hormone
E	estradiol 17-β
P	progesterone
$PGF_{2\alpha}$	prostaglandin $F_{2\alpha}$
DF	dominant follicle
NL	non-lactating
L	lactating
NA	not available
DF Dev	size of the dominant follicle at deviation
Sub Dev	size of the largest subordinate follicle at deviation
DF Peak	largest diameter of the dominant follicle
Sub Peak	largest diameter of the largest subordinate follicle
GnRH	gonadotropin releasing hormone

Introduction

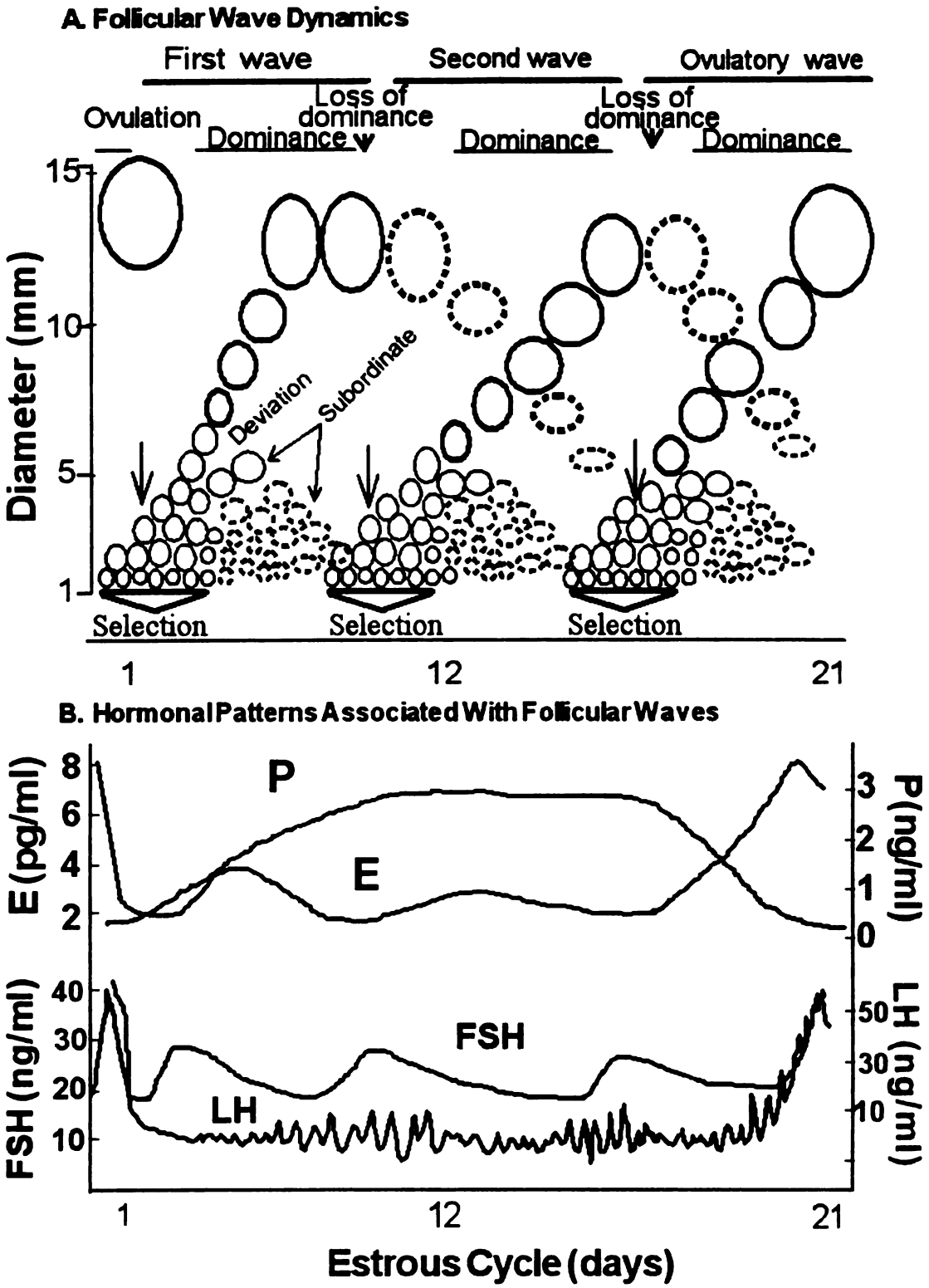
Background. The development of ovarian follicles begins during embryonic development [1]. In sheep and cattle, "nests" of primordial follicles are first observed in ovaries of 2 to 3-month old embryos [1, 4]. Primordial follicles contain immature oocytes arrested in meiosis I and surrounded by a single layer of flattened pre-granulosa cells. Unlike males that produce germ cells (sperm) throughout most of life, the final size of the pool of germ cells (oocytes) in females is established at birth. Although highly variable (0 in freemartins to 800,000), average number of primordial follicles in calves at birth is 100,000 per pair of ovaries [5]. Follicle recruitment, which is the process whereby a cohort of primordial follicles enters into a trajectory of growth [6], begins in embryos and continues until the primordial follicle pool is depleted [1]. Recruitment is initiated by the transformation of a cohort of primordial follicles into primary follicles. Primary are distinguished from primordial follicles because they have an oocytes surrounded by one complete layer of cubodial rather than squamous granulosa cells. The hormonal or growth factors that regulate recruitment are unknown. As primary follicles grow, they develop into secondary or preantral follicles. Secondary follicles consist of an oocyte serially surrounded by >2 layers of granulosa cells, a basement membrane and theca layers of cells. As secondary follicles grow, they become antral or tertiary follicles. Antral follicles are similar to secondary follicles, except they are larger and have antral cavities filled with follicular fluid. In cattle, antral follicles begin to form as secondary follicles reach from 0.14 to 0.75 mm in diameter [7, 8]. Some antral follicles continue to

grow until they reach ovulatory size, which is usually 15 to 20 mm in diameter [9]. Understanding the mechanisms that regulate development of primordial into ovulatory follicles is fundamental to the design of better therapeutic methods to improve fertility in cattle.

In cattle, ultrasound, coupled with follicle mapping, has been widely used to monitor the patterns of growth of antral follicles that are ≥ 3 mm in diameter, and to characterize development of "dominant" follicles during the estrous cycle. A dominant follicle is defined as a follicle that survives in a hormonal milieu unfit for growth of other follicles, and (or) a follicle that prevents growth of other follicles during a follicular wave [2, 10]. Based on results of ultrasound analysis from numerous laboratories, growth of antral follicles occurs in a cyclic or wave-like fashion every 7 to 10 days beginning before puberty and continuing unabated throughout most of life [2, 9, 11-13]. During an estrous cycle, there are usually two or three waves of antral follicle growth with the final or ovulatory wave ending in ovulation of the dominant follicle on day 1 of the estrous cycle (Figure 1A). During emergence (first day of wave), a cohort of several dozen antral follicles 3 to 4 mm in diameter are stimulated to continue to grow by a transient rise in serum concentrations of follicle stimulating hormone (FSH) (Figure 1A; [11, 14]. As FSH declines during the first 1 to 2 days of a wave, the growing FSH-dependent follicles in the wave undergo selection [15]. Selection is the reduction in size of a cohort of growing follicles in a wave to the species-specific ovulatory quota [16], which is one in cattle. The end of selection during a wave occurs 2 to 3 days after emergence and is associated with the following well characterized physiological and morphological phenomena: a gradual decline in serum concentrations of FSH (Figure 1B; [14, 15]),

deviation in rate of growth of a single dominant compared with other subordinate follicles (Figure 1A; [13]), enhanced estradiol production and diminished production of the small molecular weight insulin-like growth factor binding proteins (IGFBPs) by the dominant compared with other subordinate follicles [17], onset of dominance (Figure 1A; [10]), and atresia (death) of all remaining FSH-dependent subordinate follicles (Figure 1A; [15]). During dominance, while serum FSH concentrations are low, the dominant follicle, which has enhanced numbers of LH receptors compared with subordinate follicles [18, 19], becomes primarily dependent on luteinizing hormone (LH) for its continued survival [20, 21]. Based on ultrasound analysis, deviation occurs as the growth rate of the dominant follicle exceeds that of all other subordinate follicles in the wave (Figure 1A; [13]), whereas onset of dominance commences when a single dominant follicle reaches a diameter 1 to 2 mm larger than the next largest subordinate follicle, and the growth of all subordinate follicles in a wave ceases (Figure 1A; [15, 22]). As dominant follicles continue to develop during a wave, the frequency of episodic LH secretion is altered (Figure 1B;[20, 21]). A decrease in the frequency of LH secretion, which occurs during the luteal phase of an estrous cycle, leads to loss of dominance and eventual regression of non-ovulatory dominant follicles (Figure 1A;[23]). In contrast, an increase in episodic secretion of LH, which occurs coincident with the reduction in progesterone concentrations during regression of the corpus luteum, leads to development of an ovulatory dominant follicle during the follicular phase of an estrous cycle (Figure 1A; [23]). Loss of dominance not only marks the end of a follicular wave, but also coincides with emergence of the next follicular wave, and usually occurs at the time the dominant follicle ceases to grow (Figure 1A).

Figure 1. A. The dynamics of ovarian follicular waves and antral follicle turnover during the estrous cycle of cattle [2]. **B**. Hormonal patterns associated with follicular waves [3]. E = Estradiol. LH = Lutenizing Hormone. FSH = Follilcle Stimulating Hormone. P = Progesterone. Detailed explanation provided in the Introduction.


A. Follicular Wave Dynamics
First wave
Second wave
Ovulatory wave
Ovulation
Loss of dominance
Dominance
Loss of dominance
Dominance
Dominance
Diameter (mm)
15
10
5
1
Deviation
Subordinate
Selection
Selection
Selection
1
12
21
B. Hormonal Patterns Associated With Follicular Waves
E (pg/ml)
8
6
4
2
P
E
P (ng/ml)
3
2
1
0
FSH (ng/ml)
40
30
20
10
FSH
LH
LH (ng/ml)
50
30
10
1
12
21
Estrous Cycle (days)

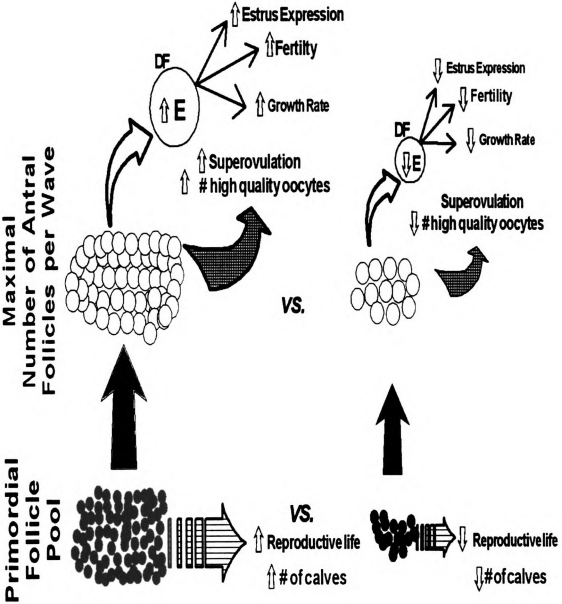
Growth of a dominant follicle can be classified into three phases based on ultrasound analysis [13]: growing, static, and regressing (Figure 1A). The growth phase begins at emergence and ends on the day the dominant follicle ceases to increase in size. The static phase is when growth of the dominant follicle ceases until a day before dominant follicle diameter begins to decrease. The static phase usually coincides with loss of dominance and emergence of a new follicle wave. The regressing phase is the last day of the static phase until the dominant follicle is no longer detectable by ultrasound.

Statement of problem. Although a single dominant follicle usually develops to ovulatory size during a follicular wave, numerous smaller antral follicles also typically develop and die during waves (Figure 1A; [13]). However, the variation in numbers of antral follicles in different follicular waves has not been carefully examined during estrous cycles of dairy heifers and cows. In addition, the physiological significance and the factors that regulate the variation in numbers of antral follicles growing during follicular waves have not been elucidated.

Speculations on the potential physiological significance of the variation in numbers of antral follicles during follicular waves in cattle. Despite cattle being single-ovulators, it is reasonable to postulate that the number of follicles in waves influences many economically important reproductive traits (Figure 2). For example, variation in number of antral follicles in waves may be associated with the variation in size of the primordial follicle pool [5, 24-26]. This finding could indicate that number of antral follicles in waves is a physiological indicator of the potential length of the reproductive lifespan of a cow (Figure 2). Perhaps, a heifer identified by ultrasound as having a

relatively high number of antral follicles in a wave also has a large primordial follicle pool, which enables her to produce more calves in her lifetime compared with cows with small pools of primordial follicles. The number of antral follicles in a wave may influence, and thus at least partially explain, the variability in responses of cattle to ovulation synchrony or superovulation protocols for embryo transfer [27-30]. For example, cattle with greater numbers of antral follicles in waves may produce more estradiol [17], which in turn may cause dominant follicles to grow faster [31] and produce more estradiol during follicular waves compared with cattle with low numbers of antral follicles in waves (Figure 2). If so, differences in number of antral follicles in waves would be expected to contribute to the variation not only in intensity of estrus expression [32], but also in the rate of development of dominant follicles and thus stage of maturation and time to ovulation after cattle are subjected to ovulation synchrony or superovulation protocols. Cattle with high numbers of antral follicles in waves may respond greater to superovulation protocols and thus produce more high quality oocytes for embryo transfer compared with cattle with low numbers of antral follicles in waves (Figure 2). Number of antral follicles in waves may also contribute to the high variability usually associated with responses of cattle to superovulation [28, 30, 33, 34]. It is highly likely that each follicle in a wave contributes to the overall hormonal and growth factor milieu both in the peripheral circulation and locally within ovaries [35]. Therefore, the hormones and growth factors produced by subordinate follicles during waves may influence not only dominant follicle development, estrus expression, and responses to superovulation, but also embryo development and overall fertility of the cow. Most important, if the variation in antral follicle number in waves is indeed linked to

Figure 2: A hypothetical model depicting the association of a high *vs* low number of antral follicles per wave with size of the primordial follicle pool, reproductive lifespan, number of calves, superovulation, estrus expression, dominant follicle growth, and fertility. E = Estradiol. DF = Dominant Follicle.


⇧Estrus Expression
⇧Fertility
DF
⇧E
⇧Growth Rate
⇧Superovulation
⇧ # high quality oocytes
Maximal Number of Antral Follicles per Wave
VS.
⇩Estrus Expression
⇩Fertility
DF
⇩E
⇩Growth Rate
Superovulation
⇩#high quality oocytes
Primordial Follicle Pool
VS.
⇧Reproductive life
⇧# of calves
⇩Reproductive life
⇩#of calves

economically important reproductive traits, then number of antral follicles in a wave could be used as a novel criterion to improve existing and perhaps develop new reproductive management systems. In addition, should number of antral follicles in waves be linked to important traits **and** controlled genetically, then ultrasound determination of number of antral follicles per wave would be a simple, novel criteria to improve breeding schemes for selection of high fertility cows. The factors that regulate the variation in number of antral follicles that develop in follicular waves are unknown, but may most likely be associated with the following: differences in serum concentrations of FSH that precede each follicular wave [14, 36-39], stage of estrous cycle when waves occur [40], variability in size of the pool of primordial preantral or antral follicles in ovaries [5, 41], environmental influences such as nutrition and temperature [42-45], and(or) genetic components [46, 47].

Hypotheses. Because very little is known about the alterations in number of antral follicles that develop in follicular waves during the bovine estrous cycle, I propose to test the following two hypotheses to complete the research requirement for my Master's degree:

I. Number of antral follicles in different follicular waves is as variable within individuals as it is amongst cattle.

II. Variation in numbers of antral follicles within follicular waves is positively correlated with each wave's corresponding FSH, inhibin-A and estradiol secretion profiles.

The specific reasons I chose these hypotheses to test are explained in Chapter I.

Approach. My general approach to address these hypotheses will be to use ultrasound and follicle mapping to monitor number of antral follicles that develop in the first, second and ovulatory waves of follicular development (Figure 1A) in heifers and cows, and to use serial blood sampling and ultrasound analysis to establish whether patterns of serum FSH concentrations are associated with number of antral follicles in waves (Figure 1B).

My thesis is in two parts: In Chapter 1, I have prepared a manuscript for submission to Biology of Reproduction describing the results of the studies in my thesis. In Chapter 2, I have included an appendix of all the follicular wave and hormone secretion data generated for each animal during my studies.

Chapter 1

Manuscript to be submitted to

Biology of Reproduction

Repeatability of Numbers of Antral Follicles in Follicular Waves in Cattle and Association with Secretion of FSH, Estradiol and Inhibin-A

Short title: Repeatability of Antral Follicle Numbers during Follicular Waves

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Keywords: number of antral follicles per wave, repeatability of follicle growth, follicle waves, FSH, estradiol, inhibin-A

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Abstract

The objective of our study is to examine the variation in antral follicle numbers that develop during follicular waves in cattle, and to determine if the variation in numbers of follicles per wave is associated with serum FSH, estradiol and inhibin-A concentrations. Non-pregnant cattle (10 months to 7 years old, n = 44) were subjected to twice daily ultrasound analysis to count number of antral follicles ≥3 mm in diameter during different follicular waves (n = 138 waves) in the same or consecutive estrous cycles or in estrous cycles spaced several months apart. The results demonstrated that: a) maximal numbers of antral follicles in different follicular waves were highly variable amongst cattle (8 to 54), but remarkably highly repeatable (0.95) within individuals; b) repeatability of the maximal number of follicles per wave in individuals was very high (0.86 to 0.95) regardless of age, season, stage of lactation, two *vs* three follicular waves per estrous cycle or technician; and c) most of the variation in maximal number of antral follicles per wave amongst cattle was attributable to follicles 3 to 5 mm in diameter. In another experiment, ultrasound analysis was used to identify cows (3 to 4 years old) that consistently had very high (29 to 44 follicles, n = 4 cows) or low numbers (11 to 18 follicles, n = 3 cows) of antral follicles per wave. Blood samples were taken at frequent intervals beginning 48 h after $PGF_{2\alpha}$ and continuing until 4 d after ovulation. Serum was subjected to FSH, estradiol and inhibin-A immunoassays. The results demonstrated that serum FSH concentrations were 50% lower ($P<0.03$) in cows with very high numbers of antral follicles per wave (34.9±1.1 follicles) *vs* cows with low numbers (15.1±0.9 follicles). However, serum concentrations of estradiol and inhibin-A were similar

between the two groups of cows. We concluded that: a) despite the high variation in antral follicle number per wave amongst cattle, a chronic compensatory mechanism must exist in ovaries to maintain growth of a constant number of antral follicles in follicular waves throughout estrous cycles of individuals, b) the variability in numbers of follicles in waves amongst cattle is probably not explained by the interaction of serum FSH with inhibin-A and estradiol concentrations, and c) factors in addition to estradiol and inhibin-A from antral follicles may control FSH secretion from the pituitary gland during follicular waves.

Introduction

Ultrasonography has firmly established that two or three follicular waves usually occur during the human menstrual cycle [48, 49] and during the bovine estrous cycle [2, 13]. However, follicular waves are best characterized in cattle [2, 12, 13, 50-52]. In cattle, follicular waves occur every 7 to 10 days beginning several months before first ovulation at puberty and continuing throughout most of life [2, 13]. Each follicular wave coincides with a transient rise in serum concentrations of follicle stimulating hormone (FSH) [14, 52]. The increase in circulating concentrations of FSH stimulates growth of a cohort of 3- to 4-mm antral follicles, which is followed by development of a single dominant ovulatory-size (15-20 mm) follicle and atresia of the remaining follicles in the original cohort [2, 13, 52]. Follicular waves synchronous with the follicular phase of an estrous cycle culminate in development of a single dominant ovulatory follicle. In contrast, follicular waves asychronous with a follicular phase are non-ovulatory [2, 13, 52]. Taken together, the wave-like growth and atresia of antral follicles, coincident with

a shrinking ovarian reserve (total number of follicles in ovaries) during aging in cattle [5, 53] and humans [25, 26], is reflective of a unique biological rhythm that may be critical for successful reproduction. However, neither the physiological significance nor the cause of the high variation in the number of antral follicles that grow during follicular waves is known. Elucidation of these questions may provide new insight into the physiological importance of follicular waves to fertility.

The variation in numbers of antral follicles during follicular waves amongst dairy heifers ranges from 11 to 42 [13]. However, the variation in maximal number of antral follicles growing during follicular waves amongst and within individual cattle of different ages has not been evaluated. We hypothesized that number of antral follicles in different follicular waves is as variable within individuals as it is amongst cattle because: a) the different follicular waves during an estrous cycle occur in markedly different hormonal milieus [54]; and b) size of the ovarian reserve, which may influence follicular growth, is highly variable amongst cattle and decreases as cattle age [5]. To test the aforementioned hypothesis, our first study was designed to examine the variation in number of antral follicles in different follicular waves amongst and within individual cattle of different ages. Also in cattle, FSH is the primary hormone that induces follicular waves [14, 52, 55], and estradiol and inhibin-A are produced by antral follicles during waves [17]. Thus, we hypothesized that the variation in number of antral follicles during follicular waves is positively associated with secretion of FSH, estradiol and inhibin-A. To test this hypothesis, the second study examined whether the variation in numbers of antral follicles per wave was positively associated with alterations in serum concentrations of FSH, estradiol and inhibin-A during a follicular wave.

Materials and Methods

Animals

Holstein heifers and lactating and non-lactating cows utilized for our studies were obtained from the Michigan State University Dairy Facility. Heifers were housed in free stalls whereas cows were housed in tie stall barns. Animals were provided feed and water ad libitum. All rations met NRC requirements, and studies were conducted during the Spring, Summer or Fall of 2001 and 2002. The All University Committee on Animal Use and Care at Michigan State University sanctioned all procedures involving cattle.

Ultrasound scanning procedure to count antral follicles

Ovaries in each cow were scanned with an Aloka SSD-900 linear array trans-rectal probe (Aloka Ultrasound, 7.5 MHz transducer, Wallingford, CT). To standardize counting of follicles, the following ultrasound scanning procedure was adopted. Each ovary was scanned from end to end to identify the position of the corpus luteum and antral follicles in ovaries. Specifically, beginning at one end of the ovary, video images for different ovarian sections were captured on a computer monitor. The location of the corpus luteum and each antral follicle $\geq$ 3 mm in each section of ovary was drawn on an ovarian map. Two separate measurements of diameter were made for each follicle and recorded next to the appropriate follicle on each ovarian map. The final diameter of each follicle was based on the average of the two separate diameter measurements. After all ovarian structures were mapped and diameters of each follicle recorded, the scanning

procedure was repeated to confirm the locations of follicles on each ovarian map. Total number of antral follicles per pair of ovaries for each animal was determined by counting the number of follicles $\geq$ 3 mm in diameter on each map for each animal.

Study 1: Validation of ultrasound scanning to count antral follicles

This study examined the variability of using ultrasound scanning to count number of antral follicles in both ovaries of individual cows within and among different ultrasound operators.

Part A. To determine the variability of counting follicles by a single ultrasound operator, the repeatability of counting the total number of antral follicles $\geq$ 3 mm in diameter in ovaries of five non-lactating 3- to 5-year-old cows on three separate occasions (spaced 10 minutes apart) twice daily (0800 and 1800) for 11 consecutive days beginning at random stages of the estrous cycle was determined. Repeatability is defined below in Statistics.

Part B. To examine the variability of counting follicles amongst ultrasound operators, the correlation of the total number of antral follicles $\geq$ 3 mm in diameter counted in ovaries during ultrasound analysis on each of 11 randomly chosen days during the estrous cycles of five non-lactating cows between two different ultrasound operators was determined. The daily ultrasound session for each cow by each operator was approximately 1 hour apart, and the results of counting follicles by each operator were undisclosed until the study ended.

Study 2: Variability and repeatability of numbers of antral follicles per wave

This study determined if number of antral follicles $\geq$ 3 mm in diameter that grow in different follicular waves during the same or consecutive estrous cycles (Parts A, B), or in estrous cycles several months apart (Part C) is repeatable in individuals.

Part A. To examine repeatability of numbers of antral follicles in different follicular waves during the same or consecutive estrous cycles, fourteen 10- to 12-month-old heifers and sixteen 4- to 7-year-old lactating (n = 9 cows) or non-lactating (n = 7) cows were subjected to ultrasound analysis. All animals were treated with two intramuscular injections of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$, 25 mg/dose of Lutalyse® Pharmacia-Upjohn Co., Kalamazoo, MI) spaced 11 days apart to induce luteolysis and synchronize occurrence of estrus. Each animal was subjected to twice daily (0800 and 1800) ultrasound analysis by the same ultrasound operator (DB) beginning at the time of the second $PGF_{2\alpha}$ injection and continuing until 2 days after spontaneous ovulation of the subsequent estrous cycle. At each ultrasound session, total number of antral follicles $\geq$ 3 mm in diameter in ovaries of each animal was determined, as described for Study 1.

Part B. This study determined repeatability of number of antral follicles in follicular waves of individual cattle using a different ultrasound operator (FJ). In this study, three heifers (13 months of age) and two lactating Holstein cows (3-5 years of age) were treated with $PGF_{2\alpha}$ and subjected to twice daily ultrasound analysis to count number of antral follicles $\geq$ 3 mm, as explained in Part A.

Part C. In this study, repeatability of number of antral follicles in follicular waves that occurred in estrous cycles spaced 3 months apart for the same four non-

lactating cows (4 to 7 years of age) was examined. Estrous cycles were either spontaneous or synchronized with two or three injections of $PGF_{2\alpha}$ spaced 11 days apart as explained above. Numbers of antral follicles $\geq$ 3 mm in diameter were counted twice daily either throughout all the different follicular waves during each estrous cycle for two cows or only during the first wave of three consecutive estrous cycles for the remaining two cows.

Study 3: Relationship of variation in maximal numbers of antral follicles per wave with the dynamics of development of dominant and subordinate follicles

This study examined whether variation in numbers of antral follicles per wave was associated with alterations in the dynamics of growth of dominant and subordinate follicles. In this study, the animals used in Study 2 (Parts A, B) were classified arbitrarily into four groups based on the maximal number of antral follicles $\geq$ 3 mm in diameter per wave. Because maximal number of antral follicles was determined for 3 to 4 waves in each animal in Study 2, individuals were classified based on their overall average for the maximal number of follicles per wave as follows: low ($\leq$15 follicles per wave; n = 30 waves, n = 9 animals), intermediate (16 to 20; n = 29 waves, n = 10 animals), high (21 to 25; n = 25 waves, n = 8 animals), and very high (>25; n = 30 waves, n = 8 animals). To determine if numbers of antral follicles per wave were associated with alterations in the dynamics of growth of dominant and subordinate follicles, we examined whether day of emergence for each follicular wave, day of deviation, length of dominance, interval between ovulations, and maximal diameter of the dominant or subordinate follicle at deviation or during dominances differed for cattle with low, intermediate, high, and very

high numbers of follicles in waves. Definitions used to characterize dominant follicle growth were: day of emergence = first day of new wave that a follicle ≥ 3 mm in diameter is detected by ultrasound; day of deviation = first day of a new wave that the largest or dominant follicle is consistently □ 1 mm greater in diameter than the next largest or subordinate follicle throughout the wave minus one day; length of dominance = interval from first day of deviation in a new wave until emergence of the next wave.

To determine if basal numbers of antral follicles per wave differed amongst the different groups of cattle, the overall average for the lowest number of antral follicles per wave was determined for animals with low, intermediate, high or very high numbers per wave.

To determine which size category of antral follicles was most variable during waves for cattle with low, intermediate, high or very high numbers of follicles per wave, the distribution of maximal numbers of follicles in each of the following different follicle size categories (3 to 3.9, 4 to 4.9, 5 to 5.9, 6 to 7.9, 8 to 10, and >10 mm in diameter) was determined for each group of animals.

Study 4: Association of patterns of FSH, estradiol and inhibin-A secretion with numbers of antral follicles that grow during follicular waves

This study determined whether the serum concentrations of FSH, estradiol and inhibin-A are positively associated with numbers of antral follicles ≥ 3 mm in diameter that develop during follicular waves.

Identification of cows with low or very high numbers of antral follicles per wave. A group (n = 25 cows) of 3- to 5-year-old lactating cows was subjected to ultrasound analysis for 2 to 3 consecutive days chosen at random during an estrous cycle to identify cows that consistently had low ($\leq$ 15 follicles) or very high (> 25 follicles) numbers of antral follicles $\geq$ 3 mm in diameter. Five animals in each group were identified. To determine numbers of antral follicles in follicular waves, estrous cycles for these ten animals were synchronized with three consecutive injections of $PGF_{2\alpha}$ spaced 11 days apart. Each animal was subjected to daily (0800) ultrasound analysis after each of the first two $PGF_{2\alpha}$ injections and twice daily (0800 and 2000) ultrasound analysis after the third $PGF_{2\alpha}$ injection. Ultrasound analysis began at the time of each injection of $PGF_{2\alpha}$ and ended 4 days after ovulation. Number of antral follicles in the first follicular wave that occurred after each $PGF_{2\alpha}$ injection was determined as explained above. Day of ovulation after each $PGF_{2\alpha}$ injection was determined based on inability of the ultrasound operator to detect the dominant ovulatory follicle during scanning.

Blood collection. Blood samples (5 ml) were removed from each animal's tail vein beginning 48 h after the third $PGF_{2\alpha}$ injection and continuing every 4 h until 24 h after ovulation. Thereafter, blood was collected every 8 h until 96 h after ovulation. Based on previous results [54], this blood sampling regimen should span the days of an estrous cycle that coincide with development of the first wave dominant follicle.

Immunoassays

Concentrations of FSH in duplicate 100-μl serum samples for each cow were determined using a previously validated heterologous RIA [54, 56, 57]. Results of the FSH RIA correlate with *in vitro* bioassay of FSH bioactivity [58]. Ovine FSH (USDA

oFSH-19-SIAFP-I-2) was used as radioiodinated tracer, bFSH (USDA bFSH-I-2) as standard, and NIDDK anti-oFSH-1 (AFP-C5288113; Rockville, MD) as antiserum. The intra-assay coefficient of variation (CV) was 10.5%, and the sensitivity of the assay was 0.03 ng/ml. Estradiol 17-β concentrations were determined in duplicate 500-μl serum samples previously extracted with ether using a modified version [59] of the commercial MAIA Kit (Polymedco Inc., Courtlandt Manor, NY). Standard curves ranged from 0.195 to 50 pg/ml (or 0.039 to 10 pg/tube), and the mean $\pm$SEM ED_{50} for standard curves (n = 4) averaged 4.8 $\pm$0.8 pg/ml. Sensitivity of the assay was 0.04 $\pm$ 0.01 pg/ml. Inter- and intra-assay (n = 4 assays) coefficients of variation (CVs) were 17.5% and 9.6% for serum samples that averaged 5.1 pg/ml, and 16.5% and 24.4% for samples (n = 4) that averaged 0.88 pg/ml. A recently validated non-radiometric two-site ELISA using highly purified 32-kDa bovine inhibin-A as standard [60] was used to determine concentrations of inhibin-A in duplicate 100-μl serum samples for each cow. Sensitivity of the inhibin assay was 15 pg/ml, and within and between plate CVs were < 11%. The inhibin-A assay does not cross react with the bovine inhibin α subunit $pro\alpha_C$, recombinant human (rh) inhibin-B, rh-activin-A, rh-activin-B, or rh-follistatin-288[60].

Statistical Analysis

Repeatability (range = 0 to 1, 1 = perfect) is defined as the proportion of the total variance that could be attributed to animal variance, which is calculated as follows: σ^2 animal/σ^2 animal + σ^2 error [61-63]. Variance components were estimated using the MIXED procedure of SAS [64].

In Study 1, repeatability or correlation analysis (range = -1 to 1, 1 = perfect positive correlation) was used to examine variability of a single or two different operators using ultrasound scanning to count follicles.

In Study 2, repeatability of numbers of antral follicles in different follicular waves during the same or consecutive estrous cycles, or in estrous cycles spaced three months apart for individuals was determined, as explained above.

In Study 3, a mixed model, repeated measures approach was used to determine whether growth of dominant and subordinate follicles during follicular waves was similar for cattle that consistently had low, intermediate, high or very high numbers of antral follicles per wave [64]. The cattle used in Parts A and B of Study 2 were used for this analysis. Main effects included: technician, groups of cattle with low, intermediate, high or very high maximal number of antral follicles per wave, lactation status, age of animals, number of follicular waves per estrous cycle, ovulatory and non-ovulatory waves, whereas dependent variables included: maximal follicle number per wave, length of dominance, interval between ovulations, and diameter of the dominant or subordinate follicles at deviation or during dominance. Also, a mixed model, repeated measures approach was used to determine whether the distribution of maximal numbers of antral follicles in different size categories was similar for cattle that consistently had low, intermediate, high or very high numbers of antral follicles per wave. Number of waves subjected to ultrasound analysis per animal was treated as a repeated measure across individual cows for analyses. Least squares means were calculated for all main effects in the model. When main effects were significant ($P<0.05$), Bonferroni *t* test was used to determine whether statistical differences existed amongst individual means [64].

In Study 4, repeatability of maximal numbers of antral follicles during the three synchronization periods was determined, as explained above. A mixed model, repeated measures approach was used to determine if serum FSH, estradiol and inhibin-A concentrations and numbers of antral follicles were different for cows with low *vs* very high numbers of antral follicles per wave [64]. Main effects included: groups of cows with low or very high numbers of antral follicles per wave, time, and an interaction of the follicle groups with time. Serum FSH, estradiol and inhibin-A concentrations and numbers of antral follicles were considered independent variables. Number of blood samples and number of follicles per wave per animal were treated as a repeated measure across individual cows. The data were aligned based on the first peak FSH concentration following ovulation. Least squares means were calculated for all main effects in the model. When main effects were significant ($P<0.05$), Bonferroni *t* test was used to determine whether statistical differences existed amongst individual means [64].

Results

Study 1: Validation of ultrasound scanning to count antral follicles

Number of antral follicles (range = 4 to 22 follicles per cow) counted at three separate ultrasound sessions spaced 10 min apart in individual animals was highly repeatable (0.97, n= 330 counts) for a single ultrasound technician. In addition, daily follicle counts (range = 6 to 25 follicles per cow) for each of the five cows during the 11-day observation period was highly correlated (correlation = 0.92, n=55 counts/operator) between the two ultrasound operators.

Study 2: Variability and repeatability of numbers of antral follicles in follicular waves amongst and within individuals

Part A: Numbers of antral follicles per wave for four representative cows that consistently had low, intermediate, high, or very high numbers of antral follicles in the different follicular waves during an estrous cycle are depicted in Figure 3. These results typified the remarkable similarity in the maximal number of antral follicles in different follicular waves of individual animals, and the great variation amongst animals. Despite the high variability in maximal number of antral follicles in waves amongst cattle (range = 11 to 54 follicles per wave), repeatability of the maximal number of antral follicles in different follicular waves during the same or consecutive estrus cycles within each individual was very high overall (0.95, n = 96 follicular waves for 30 total animals), and very high for heifers (0.92, n = 14 heifers, n = 45 waves) or cows (0.96, n = 16 cows, n = 51 waves). In addition, repeatability of basal or minimal numbers of antral follicles per wave in individuals (range = 2 to 31) was very high overall (0.85, n = 68 waves, n = 30 animals), and very high for heifers (0.84, n = 31 waves, n = 14 heifers) or cows (0.87, n = 37 waves, n = 16 cows). *Note.* The minimal number of follicles per wave was not determined for all waves because number of follicles was not determined for the last 2 to 3 days of the last wave examined in each animal. Thus, the total numbers of waves examined to determine minimal numbers of follicles per wave was less than total numbers of waves examined per animal to determine maximal numbers of follicles per wave.

The overall correlation for alterations in numbers of follicles in the right *vs* left ovary during all follicular waves for 30 animals was 0.72 ($P<0.01$).

Part B: In a separate study by another ultrasound operator, the maximal number of antral follicles per wave for five animals ranged from 9 to 39 (n = 18 waves), whereas repeatability of numbers of follicles per wave for individuals was 0.86.

Part C: The maximal number of antral follicles per wave ranged from 9 to 33 amongst four animals (n = 21 waves), whereas repeatability of numbers of follicles per wave in estrous cycles spaced three months apart was 0.90 in individuals.

Overall follicular dynamics and variability and repeatability of maximal numbers of antral follicles per follicular wave: When the 35 animals in Parts A and B of Study 2 were combined, seven (4 cows, 3 heifers) had three follicular waves while 28 (14 cows, 14 heifers) had two follicular waves during an estrous cycle. The maximal number of antral follicles $\geq$ 3 mm in diameter in follicular waves averaged 21.5 $\pm$ 0.8 (range = 8 to 54 follicles per wave) and was similar for heifers (20 $\pm$ 1.1, range = 8 to 42 follicles per wave, n = 17 animals) and cows (22.8 $\pm$ 1.3, range = 11 to 54, n = 18). Nevertheless, cows had longer ($P<0.05$) intervals between ovulations (23.7 $\pm$ 0.6 *vs.* 21.3 $\pm$ 0.8 d), larger ($P<0.05$) dominant follicles (15.8 $\pm$ 0.3 *vs* 14.7 $\pm$ 0.5 mm), and longer ($P<0.05$) periods of dominance (7.6 $\pm$ 0.3 *vs* 6 $\pm$ 0.6 d) compared with heifers.

When animals in Parts A and B of Study 2 and Study 4 (described below) were combined (n = 44 animals, n = 138 waves), maximal number of antral follicles per wave ranged from 8 to 54 amongst animals, whereas repeatability of maximal number of follicles per wave for individuals was 0.95 (Table 1). In addition, repeatability of maximal number of antral follicles per wave in individuals was very high (0.86 to 0.95) regardless of age of cattle, season of year, numbers of follicular waves per estrous cycle, stage of lactation, or technician (Table 1).

Study 3: Relationship of number of antral follicles per wave and the dynamics of development of dominant and subordinate follicles

Distribution of numbers of heifers or cows with two or three follicular waves per estrous cycle in the low, intermediate, high or very high classifications for numbers of antral follicles per wave is shown in Table 2. The overall distribution of heifers and cows in each follicle number per wave classification was equally distributed ($P > 0.10$).

Ages of cattle, stage of lactation, length of dominance, interval between ovulations, day of emergence, day of deviation, largest diameter of the dominant and subordinate follicles during dominance, and diameter of the dominant and subordinate follicles at deviation were similar ($P > 0.10$) for cattle with low, intermediate, high, or very high numbers of follicles per wave (data not shown).

Basal numbers of antral follicles per wave were highest ($P<0.05$) in cattle with very high numbers of follicles per wave (19.7 ± 1.2), greater ($P<0.05$) in cattle with very high or high (11.3 ± 1) numbers of follicles per wave compared with cattle with intermediate (7.3 ± 1) or low (6.2 ± 0.9) numbers of follicles per wave, and similar ($P>0.10$) for cattle with low or intermediate numbers of follicles per wave.

The distribution of maximal numbers of antral follicles per wave in the 5 to 5.9, 6 to 7.9, 8 to 9. 9 or >10 mm in diameter categories was similar ($P > 0.10$) for cattle with different numbers of antral follicles per wave, except that cattle with very high numbers of follicles per wave had greater ($P<0.05$) numbers of follicles 5 to 5.9 mm in diameter compared with cattle with low numbers of follicles per wave (Table 3). In contrast, numbers of follicles 3 to 3.9 and 4 to 4.9 mm in diameter were 2- to 3-fold higher

($P<0.05$) in cattle with high or very high *vs* low or intermediate numbers of antral follicles per wave.

Study 4: Association of patterns of FSH, estradiol and inhibin-A secretion with numbers of antral follicles that grow during follicular waves

Of the 10 cows subjected to ultrasound analysis in Study 4, one cow predicted to have a low number of antral follicles expired while another developed a large (>30 mm) follicular cyst, and one cow predicted to have a very high number of antral follicles developed leg problems. These three cows were excluded from blood sampling, but the latter two cows were included in determination of repeatability. Despite high variability in number of antral follicles in waves amongst cattle, repeatability of maximal number of antral follicles in three consecutive estrous cycles was very high for individuals (0.93, n = 24 waves for 9 cows) and similar to the results of Study 2.

In the seven cows used to examine the association of serum FSH, estradiol and inhibin-A concentrations to numbers of follicles per wave (Figure 4), the maximal number of antral follicles $\geq$ 3 mm in diameter per follicular wave for the three cows predicted to have a low number of antral follicles averaged 15.1 ± 0.9 follicles per wave (n = 10 follicular waves, range = 11 to 18, age = 4 years old), whereas the four cows predicted to have a very high number of antral follicles averaged 34.9 ± 1.1 follicles per wave (n = 10 follicular waves, range = 29 to 44, ages = 3-4 years old).

Because of multiple large peaks of FSH secretion for each cow during the blood-sampling period (data not shown), FSH values were aligned for each cow for statistical analysis based on the first peak FSH concentration after ovulation. Ovulation occurred an average of 4 h before the first peak FSH concentration after ovulation. Follicle wave

emergence occurred coincident with ovulation, and deviation and development of dominant follicles occurred an average of 63 h after the first peak FSH concentration after ovulation.

Serum FSH concentrations varied ($P<0.001$) similarly during the first follicular wave for cows with low *vs* very high numbers (Figure 4). However, serum FSH concentrations were nearly 50% higher ($P<0.03$) throughout the treatment period in cows with low (0.73 ± 0.06 ng/ml) *vs* very high numbers of antral follicles (0.50 ± 0.05 ng/ml). Serum concentrations of estradiol and inhibin-A were unaltered ($P>0.10$) during the treatment period (Figure 4). Also, in contrast to FSH, serum concentrations of estradiol and inhibin-A were similar for cows with low *vs* very high numbers of antral follicles per wave (Figure 4).

Discussion

The results of our study demonstrate for the first time that numbers of antral follicles that grow during different follicular waves is very highly repeatable in individual cattle. In support of repeatability of antral follicle growth in individuals, number of antral follicles is similar in individual humans from one menstrual cycle to the next [41]. In addition, numbers of antral follicles that grow following serial ultrasound-guided needle aspiration of antral follicles every 7 to 10 days is highly repeatable in individual cattle [61, 65]. Although responsiveness to superovulation treatments is not repeatable in cattle [28-30, 66], the negative effect of dominant follicles per se on follicular growth [67, 68], coupled with timing of superovulation injections relative to emergence of

follicular waves, may explain the absence of repeatability of responsiveness to superovulation in cattle. The high repeatability of follicle growth in individual cattle in our study was maintained despite striking differences in hormonal milieus associated with the different waves during estrous cycles [54], ages of cattle, numbers of follicular waves per estrous cycle, stages of lactation, and season of year. Repeatability of antral follicle growth was also high when a different technician conducted the ultrasound analyses, thus eliminating technician artifact as the cause of high repeatability. Interpretation of our results, however, is somewhat limited because it was not established definitively whether repeatability of antral follicle growth during waves remained high throughout the reproductive lifespan of individual cattle. Nevertheless, the average numbers of antral follicles per follicular wave were similar for 10- to 12-month-old heifers compared with 3- to 7-year-old cows in our study. In addition, previous reports indicate that despite the steep decrease in numbers of primordial follicles during aging, numbers of antral follicles in ovaries remain relatively constant until cattle are 9 to 10 years old [5, 53] and humans are 35 to 38 years old [24, 41, 69-72]. Thereafter, coincident with the marked exhaustion of primordial follicles, the numbers of antral follicles decrease in cattle [5, 53] and humans [24, 41, 69-72]. These observations, coupled with the remarkably high repeatability of antral follicle number per wave in individuals observed in our study, imply that a chronic intraovarian compensatory mechanism may exist to maintain a nearly constant rate of antral follicle growth as numbers of primordial follicles decline during the lengthy reproductive lifespan of single-ovulating species such as cattle and perhaps humans. Whether this compensatory

mechanism is similar to the acute compensatory mechanism that maintains the species specific ovulation rate after unilateral ovariectomy [73-75] is unknown.

Classical histological studies in cattle [5] and other species including sheep [76], rats [77], mice [78], and humans [26] demonstrate that numbers of primordial follicles are highly variable within species at birth. Because the ovarian reserve, which is comprised primarily of primordial follicles, is depleted during aging and never replenished [5, 26, 69, 76, 78], numbers of the different follicle types in the ovarian reserve vary greatly in individuals throughout reproductive life. In support of the high variability in size of the ovarian reserve, histological, laparoscopic, and ultrasound analyses of ovaries of animals of similar ages show that numbers of preantral and antral follicles are highly variable in prepubertal or adult cattle [5, 13, 53, 79], sheep [76], rats [77], mice [78], non human primates [80], and humans [26, 72]. The results of our study, coupled with another report [13], clearly show that numbers of antral follicles that grow during follicular waves in cattle are also highly variable. In cattle, higher numbers of preantral or antral follicles are positively linked to fecundity [5, 81], shorter calving intervals [82], and a higher percentage of normal oocytes [53] and number of transferable embryos [79]. In cattle and humans, higher numbers of antral follicles are positively associated with an increased responsiveness to gonadotropin treatments during superovulation [30, 79, 83-85], a larger number of oocytes recovered for *in vitro* fertilization [83, 85], and higher pregnancy rates following *in vitro* fertilization [79, 83, 85]. The high repeatability of numbers of antral follicles in waves of individuals, coupled with the high variation in follicular growth amongst cattle, enable use of ultrasound to identify reliably groups of cattle that consistently have low, intermediate,

high or very high numbers of antral follicles per wave. Consequently, future studies will take advantage of this important finding and examine the relationship of the variation in numbers of antral follicles in waves amongst cattle to size of the ovarian reserve, reproductive lifespan, superovulation and fertility.

Cattle, like humans, are a single ovulating species. Consequently, the selection process during follicular waves usually results in development of a single dominant follicle regardless of numbers of antral follicles in waves. This raises the interesting question of whether the variability in numbers of 3 to 5 mm antral follicles during waves, which is the primary size category of follicles that varied during follicular waves in our study, affects subsequent growth and function of dominant follicles. Results of our study, however, show that serum estradiol and inhibin-A concentrations, and the dynamics of development of dominant follicles during follicular waves, including emergence, deviation, maximal diameter and length of dominance, were unaltered by variability in numbers of antral follicles in waves. This surprising finding implies that numbers of antral follicles per wave may not affect development or function of dominant follicles. Nevertheless, our study focused primarily on the first half of the first follicular wave during an estrous cycle, which is non-ovulatory. Thus, secretion of FSH, estradiol and inhibin-A were not evaluated throughout the first follicular wave or during an ovulatory wave when estradiol secretion is maximal [54]. Future studies will, therefore, be necessary to determine if variation in numbers of antral follicles per wave alters dominant follicle growth and function.

The mechanisms that regulate the variation in numbers of antral follicles that grow during follicular waves are unknown. Moreover, the putative chronic

compensatory mechanism that maintains growth of a highly repeatable number of antral follicles during waves as the ovarian reserve is depleted during aging in gonadally intact individuals remains undefined. It is well established that FSH is a key hormone involved in growth of antral follicles. In support of the role of FSH in antral follicle growth, serum FSH concentrations peak prior to follicular waves in humans [49] and cattle [14, 54], and inhibition of FSH secretion in cattle blocks occurrence of follicular waves [55, 86]. Studies in hypophysectomized rats [31] and genetic deletion studies in mice [87] show clearly that FSH is required for development and differentiation of antral follicles in laboratory species. Moreover, enhanced secretion of FSH after removal of one ovary causes acute compensatory ovarian hypertrophy in cattle [88-90] and other species, including rats [77, 91], swine [92], rabbits [93], hamsters [94], and guinea pigs [95]. However, these results are controversial in cattle [96] and rats [97]. Nevertheless, despite removal of half of the ovarian reserve after unilateral ovariectomy of the aforementioned species, the species specific ovulation rate is maintained [73-75] or enhanced [96, 98]. Taken together, these observations imply that FSH has important roles not only in regulation of the variation in growth of antral follicles during follicular waves, but also perhaps in the intraovarian compensatory mechanisms that maintain growth of a constant number of antral follicles during follicular waves.

The reason several distinct peaks of serum FSH concentrations were observed in our study and by others [38] during the first follicular wave of the estrous cycle rather than a single transient increase in FSH [14, 15, 54] is unknown. One possible explanation for this disagreement among laboratories is that values in our study were aligned relative to the first FSH peak after ovulation, while others align data relative to

the preovulatory LH surge [15], emergence of follicles [14, 99] or estrus [15, 54, 55, 100]. In addition, lactating cows rather than heifers were used in our study compared with others [14, 15, 54, 55, 99].

Alterations in serum concentrations of inhibin-A and estradiol in our study were similar to previous reports for changes in serum concentrations of inhibin-A [60, 101] and estradiol [15, 54] during the first wave of follicular growth in cattle. Nevertheless, in contrast to our expected results, serum FSH concentrations were inversely rather than positively associated with numbers of antral follicles in follicular waves throughout the blood-sampling period. Equally surprising was the finding that serum concentrations of the negative feedback hormones, inhibin-A and estradiol, were similar for cows with low *vs* very high numbers of antral follicles per wave. These observations imply that factors other than FSH and its chief feedback hormones, inhibin-A and estradiol, could have a role in regulation of both FSH secretion and numbers of follicles growing during waves. Because numbers of primordial, preantral and antral follicles in ovaries are positively associated [5, 27, 53], it is likely that the variation in numbers of follicles per wave reflects relative size of the ovarian reserve. Thus, the high serum FSH concentrations in animals with low numbers of follicles per wave may be associated with ovarian aging. In support of this possibility, serum FSH is higher in women [102] and cows [103] compared with their younger counterparts. Factors that regulate variation in antral follicle numbers during waves may not only include size of the ovarian reserve, differential responsiveness of antral follicles to FSH [104], differential secretion of hormones and growth factors that alter FSH responsiveness such as LH [31] or IGF-1 [105], and genetic mechanisms [46, 104], but also growth factors that regulate

recruitment and growth of preantral follicles such as anti-mullerian hormone [106-109], *kit* ligand [110-112], activin [113], and GDF-9 [110, 114, 115]. Moreover, from a neuroendocrine viewpoint, our findings imply that estradiol and inhibin-A may not be the only feedback factors that regulate secretion of FSH during follicular waves. Rather, control of FSH secretion during follicular waves may involve other follicular factors such as activin, which stimulates FSH secretion; follistatin, which inhibits activin action; or inhibin-B, which inhibits FSH secretion [116]. In addition, differential secretion of GnRH could also explain our results. Therefore, classification of cattle based on numbers of antral follicles per wave should provide a novel model to elucidate the role of the aforementioned factors in regulation of FSH secretion and the dynamics of follicular growth in future studies.

In summary, the most significant findings in our study demonstrated that: a) numbers of antral follicles in different follicular waves are highly repeatable (0.95) within individuals, but highly variable amongst animals, and b) serum FSH concentrations are inversely associated whereas estradiol and inhibin-A are not associated with numbers of antral follicles per wave. Based on these results, we conclude that: a) despite the high variation in number of antral follicles in different waves amongst cattle, a chronic compensatory mechanism must exist in ovaries to maintain growth of a constant number of antral follicles in different follicular waves throughout estrous cycles of individuals, b) the variability in numbers of follicles in waves amongst cattle is probably not explained by the interaction of serum FSH with inhibin-A and estradiol concentrations, and c) factors in addition to estradiol and inhibin-A may have a role in regulation of FSH secretion from the pituitary gland during follicular waves.

Figure 3. Alterations in total numbers of antral follicles (≥ 3mm) during the first and ovulatory follicular waves of an estrous cycle and during the early stages of the first follicular wave of the next estrous cycle of four representative Holstein cows with low (< 15), intermediate (15 to 20), high (21 to 25) or very high (> 25) numbers of antral follicles per follicular wave. Each bar represents the average for the total number of antral follicles in both ovaries for a single cow determined at each of two daily ultrasound sessions (0800, 1800). * Above bar = maximal number of follicles in each wave. Arrows indicate ovulation. Lines represent changes in diameter of the dominant and the largest subordinate follicle for the first and ovulatory wave. DNF = Dominant non-ovulatory follicle, DOF = Dominant ovulatory follicle, SF = Largest subordinate follicle.

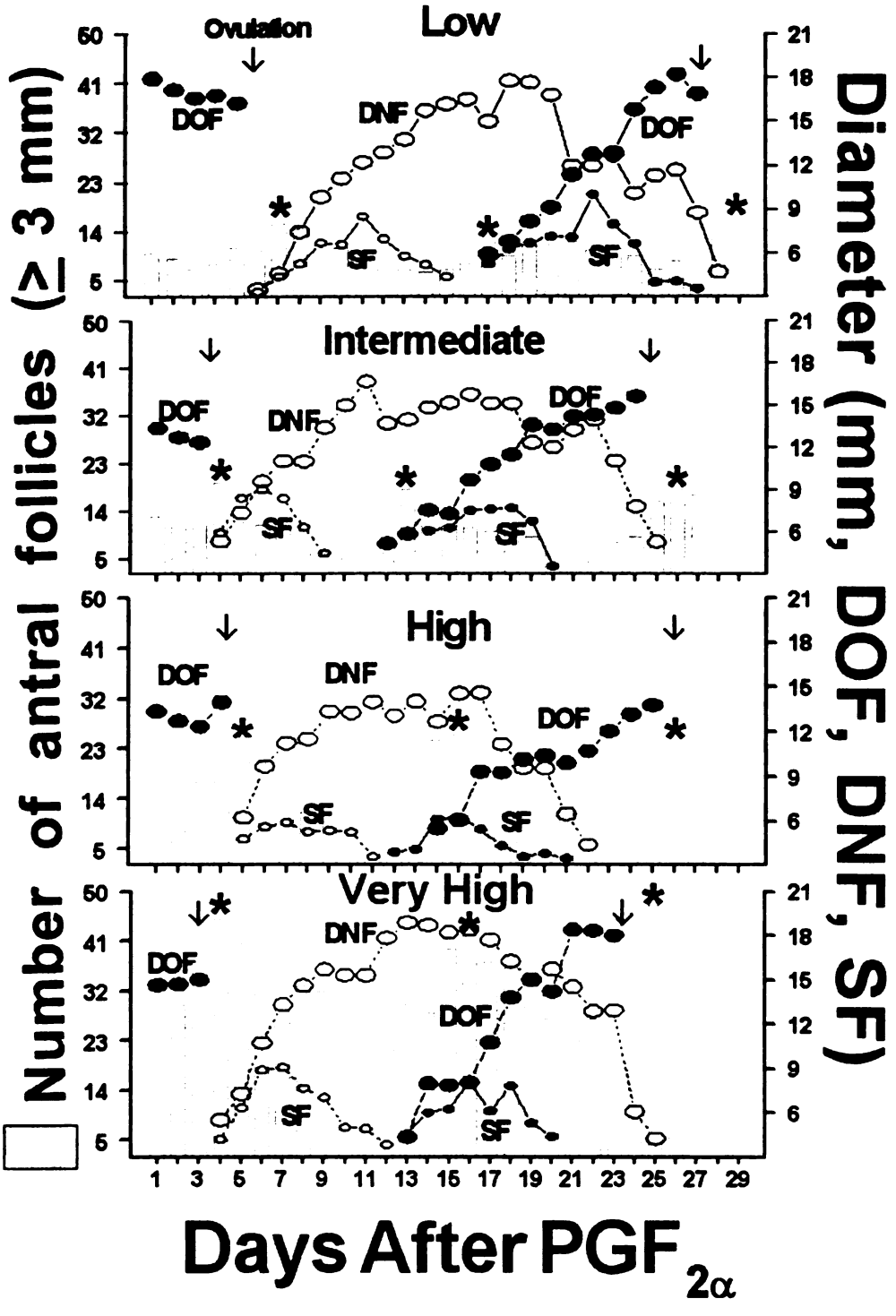

Low
Ovulation
Intermediate
High
Very High
DOF
DNF
SF
Number of antral follicles (≥ 3 mm)
Diameter (mm, DOF, DNF, SF)
Days After PGF2α

Figure 4. Serum hormone profiles, dominant follicle growth, and numbers of antral follicles for Holstein cows that consistently had low (< 15 follicles per wave, n = 3 cows, open bars or circles) *vs* very high (>25 follicles per wave, n = 4 cows, solid bars or circles) numbers of antral follicles during follicular waves. Cows with low or very high numbers of antral follicles per wave were identified by ultrasound analysis, as explained in detail in Methods. Blood sampling began 48 h after a $PGF_{2\alpha}$ injection and continued every 4 h until 24 h after ovulation. Thereafter, blood was collected every 8 h until 96 h after ovulation. Data (including ultrasound results) were aligned relative to the first peak FSH concentration that occurred within 12 h after ovulation. In the upper panel, circles represent average (±SEM) concentrations of FSH for the three cows with low (○) *vs* the four cows with very high (●) numbers of follicles per wave. In the second panel, circles represent average (±SEM) estradiol concentrations for cows with low (○) *vs* very high (●) numbers of antral follicles per wave, whereas triangles represent average (±SEM) inhibin-A concentrations for cows with low (□) *vs* very high (■) numbers of follicles per wave. In the third panel, circles represent the average (±SEM) size of the largest and presumed dominant follicle for cows with low (○) *vs* very high (●) numbers of antral follicles per wave. In the bottom panel, bars represent the average (±SEM) for the total number of antral follicles ≥ 3 mm in diameter in both ovaries for the three cows with low (□) *vs* the four cows with high (■) numbers of antral follicles per wave. The arrow in the first panel indicates that ovulation was detected an average of 4 h before the first peak FSH concentration after ovulation. In the third panel, follicle wave emergence occurred coincident with the day of ovulation, and the arrow depicts deviation in growth of

dominant *vs* the largest subordinate follicles (not shown), which occurred an average of 63 h after the first peak FSH concentration after ovulation.

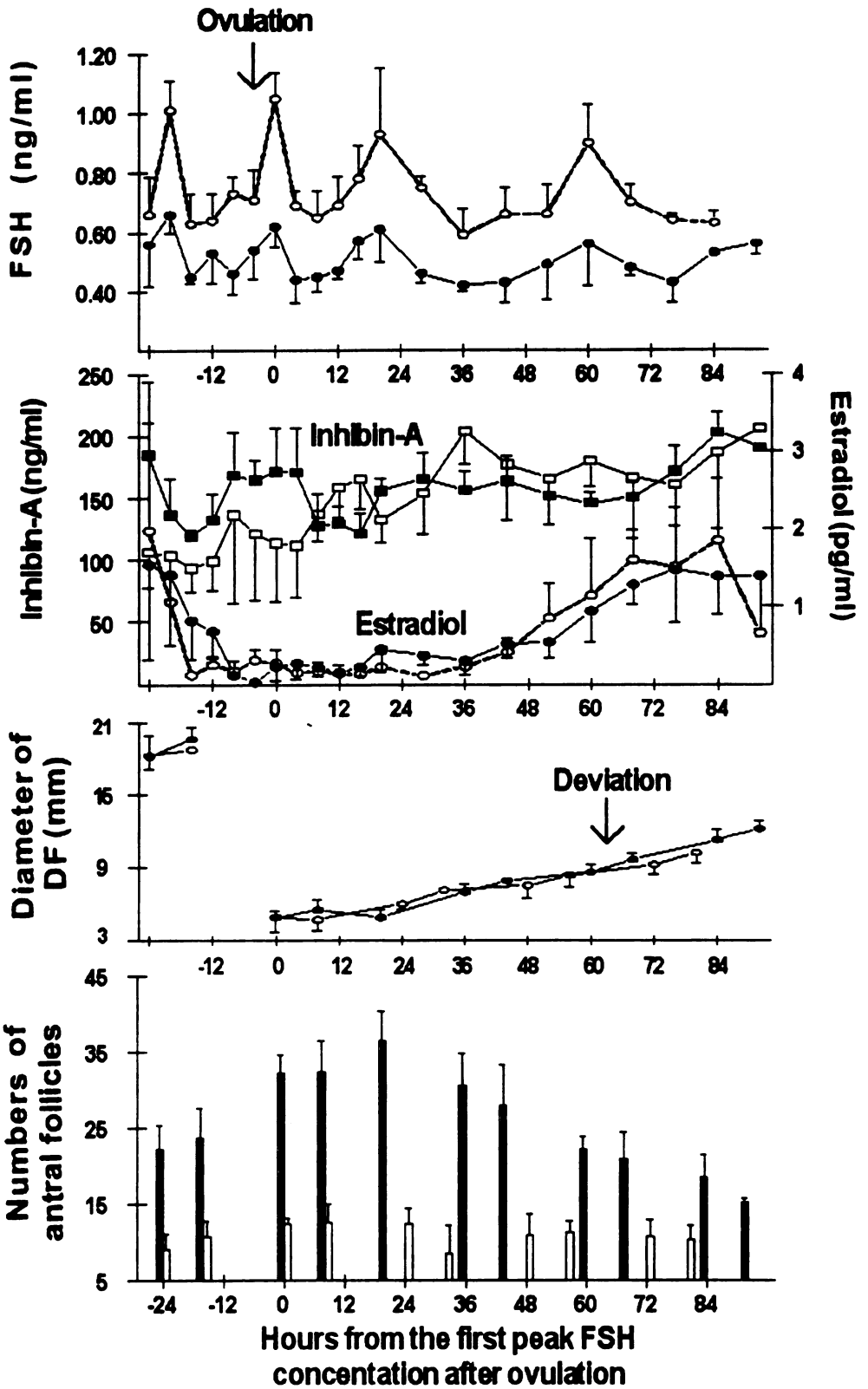

Ovulation
FSH (ng/ml)
1.20
1.00
0.80
0.60
0.40
-12
0
12
24
36
48
60
72
84
Inhibin-A(ng/ml)
250
200
150
100
50
Inhibin-A
Estradiol
Estradiol (pg/ml)
4
3
2
1
Diameter of DF (mm)
21
15
9
3
Deviation
Numbers of antral follicles
45
35
25
15
5
-24
Hours from the first peak FSH concentation after ovulation

Table 1. Effect of age, season, number of follicular waves per estrous cycle, lactational status, and technician on repeatability of maximal numbers of antral follicles (□ 3mm in diameter) per follicular wave.[A]

	Number of animals	Numbers of Waves	Range of numbers of antral follicles per follicular wave	Repeatability
Season				
Spring	5	18	8 - 39	0.86
Summer	30	96	11 - 54	0.95
Winter	9	24	12 - 38	0.94
Number of follicular waves per estrous cycle[B]				
Two	28	86	8 - 54	0.93
Three	7	28	11 - 44	0.95
Lactational Status				
Lactating	20	58	12 - 44	0.94
Non-lactating	24	80	8 - 54	0.93
Technician				
DB	39	120	11 - 54	0.95
FJ	5	18	8 - 39	0.86
Age				
Heifers[C]	19	56	8 - 42	0.92
Cows	25	82	11 - 54	0.96
Overall	44	138	8 - 54	0.95

[A] Cattle from Study 2 (Parts A and B) and Study 4 were used for this analysis.
[B] Cattle from Study 2 (Parts A and B) were used for this analysis
[C] Heifers were 10 to 12 months old, whereas cows were 3 to 7 years old.

Table 2. Distribution of numbers of heifers or cows with two or three follicular waves per estrous cycle in the low (< 15 follicles), intermediate (15-20 follicles), high (21-25 follicles), or very high (> 25 follicles) numbers of antral follicles (≥ 3 mm in diameter) per follicular wave classifications[1].

	Classification of cattle with different numbers of antral follicles per follicular wave			
	Low	Intermediate	High	Very High
Number of cattle with two follicular waves per estrous cycle:	6	9	7	6
Heifers	5	3	4	3
Cows	1	6	3	3
Number of cattle with three follicular waves per estrous cycle:	3	1	1	2
Heifers	1	1	0	1
Cows	2	0	1	1
Overall:	9	10	8	8
Heifers	6	4	4	4
Cows	3	5	4	5

[1] Holstein heifers (n = 18, 10 to 12 months of age) and cows (n = 17, 4 to 7 years of age) were given two injections of $PGF_{2\alpha}$ spaced 11 days apart to synchronize occurrence of estrous cycles. Each animal was subjected to twice daily ultrasound analysis (0800 and 1800) beginning at the time of the second $PGF_{2\alpha}$ injection and continuing until 2 days after the spontaneous ovulation of the subsequent estrous cycle. At each ultrasound session, total number of antral follicles ≥ 3 mm in diameter in ovaries of each animal was determined. Each animal was classified based on the average of the maximal numbers of antral follicles for 3 or 4 follicular waves.

Table 3. Distribution of maximal numbers of antral follicles per follicular wave in different follicle size categories for cattle that consistently had low (< 15 follicles), intermediate (15 to 20 follicles), high (21 to 25 follicles), or very high (> 25 follicles) numbers of antral follicles (≥ 3 mm in diameter) per follicular wave[1].

Numbers of antral follicles	N[2]	Overall numbers of antral follicles per wave	N[3]	Size of follicles (mm) 3-3.9	4-4.9	5-5.9	6-7.9	8-9.9	>10
Low[4]	9	12.8 ± 1.5[a,5]	30	10.9 ± 0.6[a]	2.9 ± 0.3[a]	3.1 ± 0.2[a]	2.9 ± 0.3	1.6 ± 0.2	1.5 ± 0.1
Intermediate	10	16.8 ± 1.7[b]	29	14.6 ± 0.5[ab]	4.1 ± 0.3[b]	3.6 ± 0.2[ab]	3.0 ± 0.2	1.6 ± 0.2	1.6 ± 0.1
High	8	22.1 ± 1.9[c]	25	19.0 ± 0.7[b]	5.4 ± 0.5[c]	3.5 ± 0.3[ab]	2.8 ± 0.2	1.5 ± 0.1	1.4 ± 0.2
Very High	8	33.8 ± 1.5[d]	30	30.0 ± 2.8[c]	6.2 ± 0.4[c]	4.1 ± 0.2[b]	3.4 ± 0.4	1.7 ± 0.2	1.6 ± 0.2

[1] Holstein heifers (n = 18, 10 to 12 months of age) and cows (n = 17, 4 to 7 years of age) were given two injections of $PGF_{2\alpha}$ spaced 11 days apart to synchronize occurrence of estrous cycles. Each animal was subjected to twice daily ultrasound analysis (0800 and 1800) beginning at the time of the second $PGF_{2\alpha}$ injection and continuing until 2 days after the spontaneous ovulation of the subsequent estrous cycle. At each ultrasound session, total number of antral follicles ≥ 3 mm in diameter in ovaries of each animal was determined. Each animal was classified based on the average of the maximal numbers of antral follicles for 3 or 4 follicular waves.

[2] N = number of animals

[3] N = number of follicular waves.

[4] Number of cattle and total number of waves examined by ultrasound for each antral follicle growth classification follows: Low (n= 9 animals, n= 30 waves), Intermediate (n= 10 animals, n= 29 waves), High (n= 8 animals, 25 waves), Very High (n= 8 animals, n= 30 waves).

[5] Means within a column with uncommon superscripts are significantly (P< 0.05) different.

Chapter 2

Appendix of Data for Individual Animals

In Study 2 (Parts A and B) and Study 4

LIST OF TABLES

LIST OF FIGURES

Part A

Tables and figures for individual animals in Study 2A

Definitions and descriptions of tables for each animal in Study 2A.

Table A shows the ages, milking status and genetic background for each animal, Table B shows follicular wave dynamics, and Table C shows the distribution of maximal numbers of antral follicles per follicular wave in different follicle size categories for each animal.

Table A. Definitions

NL	non-lactating
L	lactating
NA	not available

Table B. Definitions

Classification

1	low
2	intermediate
3	high
4	very high

Dominant Follicle Growth

DF	dominant follicle
DF Dev	size of the dominant follicle at deviation
Sub Dev	size of the largest subordinate follicle at deviation
DF Peak	largest diameter of the dominant follicle
Sub Peak	largest diameter of the largest subordinate follicle

Definitions and description for figures for each animal in Study 2A.

Alterations in total numbers of antral follicles ($\geq$ 3mm) during the first and ovulatory follicular waves of an estrous cycle and during the early stages of the first follicular wave of the next estrous cycle of each animal in this study. Each bar represents the average for the total number of antral follicles in both ovaries determined at each of two daily ultrasound sessions (0800, 1800). * Above bar = maximal number of follicles in each wave. Arrows indicate ovulation. Lines represent changes in diameter of the

dominant and the largest subordinate follicle for the first and ovulatory wave. DNF = Dominant non-ovulatory follicle, DOF = Dominant ovulatory follicle, SF = Largest subordinate follicle.

Table 4. A: Animal information for cow ID 3547.

Animal ID	Age (yr.)	Milking Status	Sire	Dam	Dam's Sire
3547	5	NL	Margene Blackstar Fred	Michigan CO Felicia 3167	Zee-Cal Commotion

Table 4. B: Follicle wave dynamics for cow ID 3547.

Wave #	Maximal Number of Antral Follicles	Baseline Number of Antral Follicles	Day of Emergence (d)	Day of Deviation (d)	Period of Dominance(d)	Classification	Dominant Follicle Growth (mm)			
							DF Dev	SUB Dev	DF Peak	SUB Peak
1	12	4	1	2	8	1	4.85	4.83	16.35	3.76
2	13	6	10	10	3	1	3.98	3.25	15.56	3.5
3	11	4	13	13	10	1	8.45	3.1	15.5	3.2
4	12	NA	NA	NA	NA	1	NA	NA	NA	NA

Table 4. C: Distribution of maximal number of antral follicles by size for cow ID 3547.

Wave #	Size of follicles (mm)					
	3 - 3.9	4 - 4.9	5 - 5.9	6 - 7.9	8 - 9.9	>10
1	12	4	4	2	2	1
2	11	2	2	2	1	1
3	8	3	2	2	1	1

Figure 5: Alterations in total numbers of antral follicles (≥ 3mm) during the first and ovulatory follicular waves of an estrous cycle and during the early stages of the first follicular wave of the next estrous cycle for cow ID 3547.

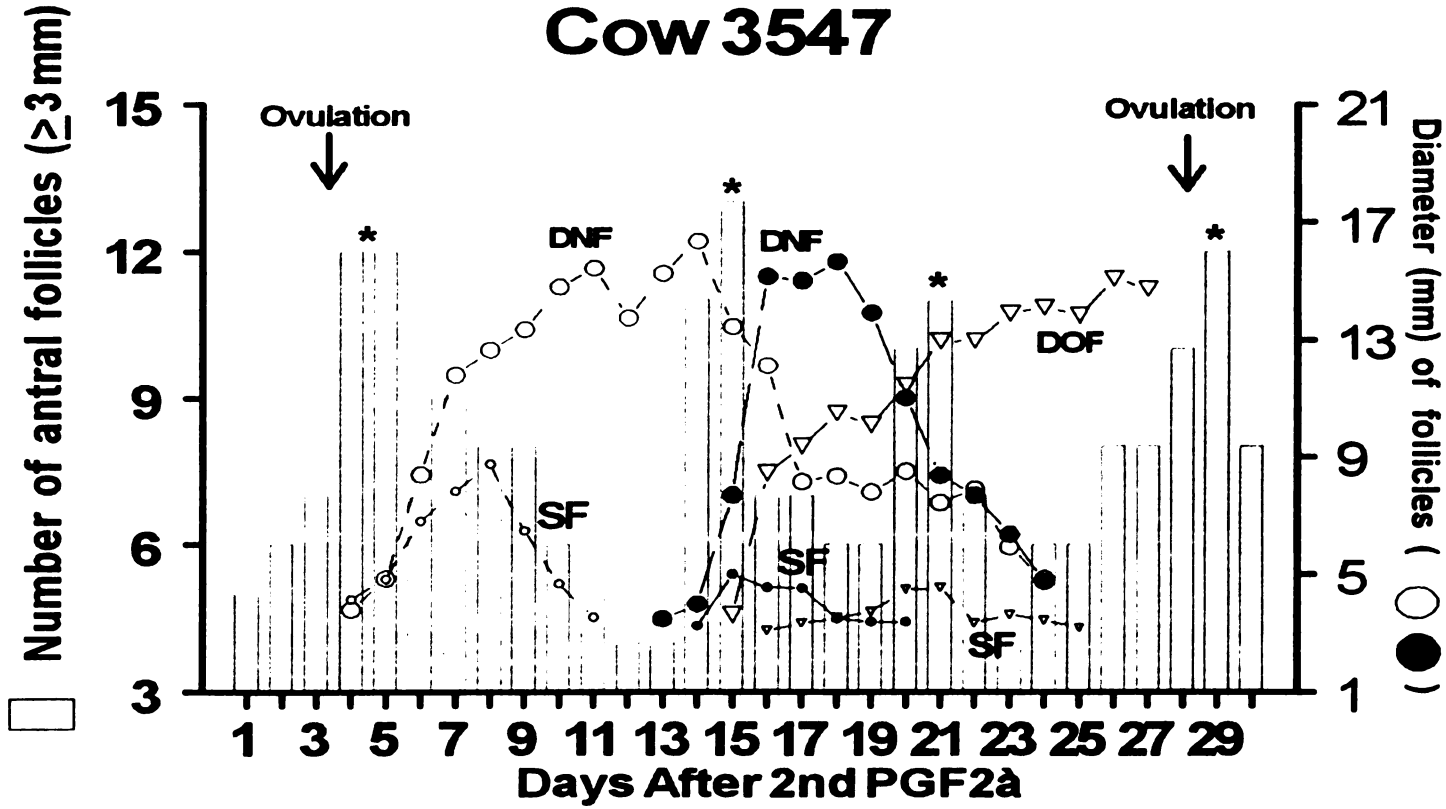


Table 5. A: Animal information for cow ID 3612.

Animal ID	Age (yr.)	Milking Status	Sire	Dam	Dam's Sire
3612	4	L	Ricecrest Tesk Terry	Michigan Bellwood Freda 3412	Maizefeild Bellwood

Table 5. B: Follicle wave dynamics for cow ID 3612.

Wave #	Maximal Number of Antral Follicles	Baseline Number of Antral Follicles	Day of Emergence (d)	Day of Deviation (d)	Period of Dominance(d)	Classification	Dominant Follicle Growth (mm)			
							DF Dev	SUB Dev	DF Peak	SUB Peak
1	15.5	6.5	1	2	8	1	4.6	4.38	17.75	3.86
2	12	7	12	13	8	1	6.8	6.28	18.18	4.05
3	16	NA	NA	NA	NA	1	NA	NA	NA	NA

Table 5. C: Distribution of maximal number of antral follicles by size for cow ID 3612.

Wave #	Size of follicles (mm)					
	3 - 3.9	4 - 4.9	5 - 5.9	6 - 7.9	8 - 9.9	>10
1	11	4	4	3	2	2
2	9	3	3	4	1	2

Figure 6: Alterations in total numbers of antral follicles (≥ 3mm) during the first and ovulatory follicular waves of an estrous cycle and during the early stages of the first follicular wave of the next estrous cycle for cow ID 3612.

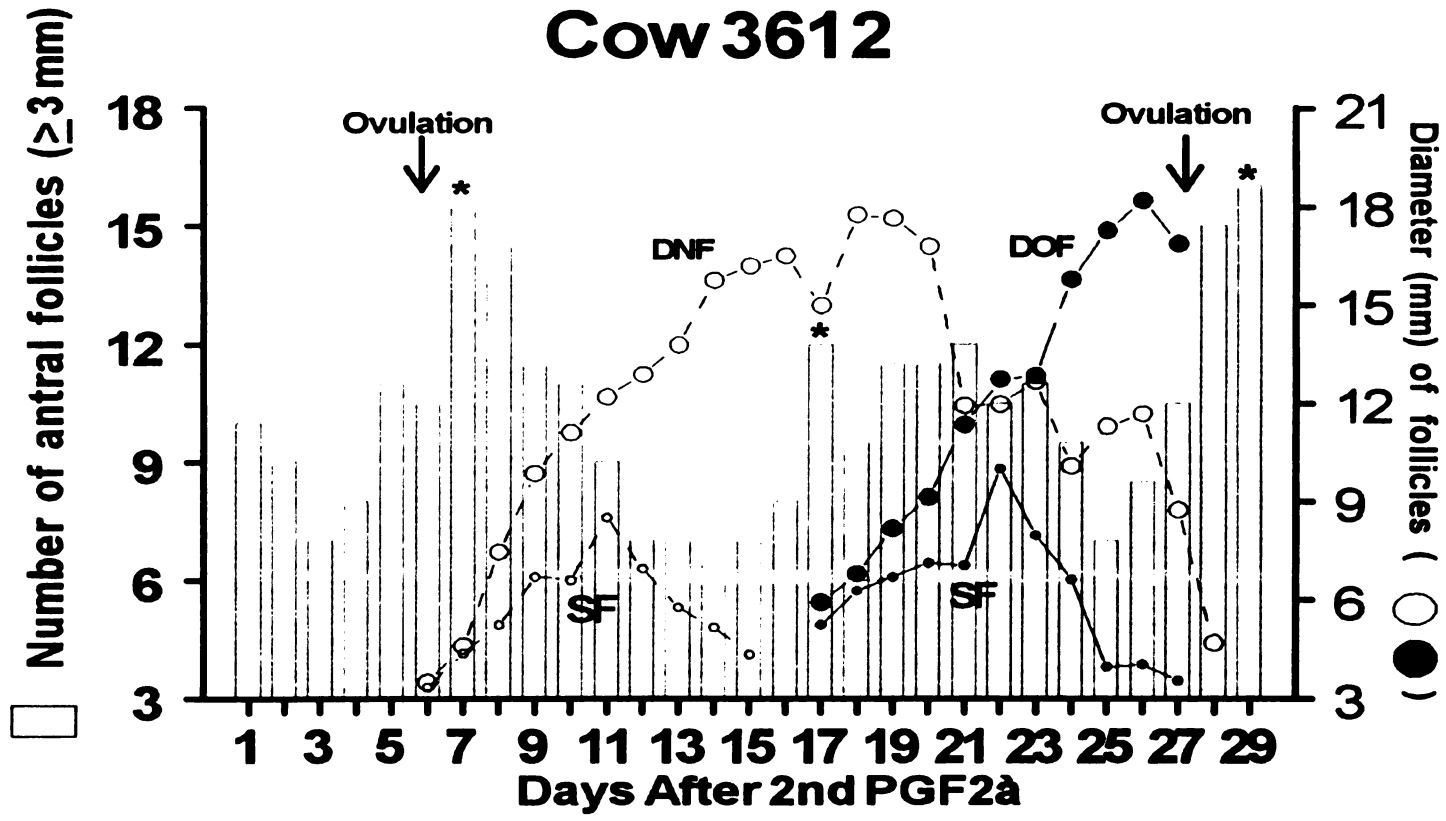


Table 6. A: Animal information for cow ID 3713

Animal ID	Age (yr.)	Milking Status	Sire	Dam	Dam's Sire
3713	4	L	Moroville Mascot Brock	Michigan Lan Phylis 3396	Lexy-Lane Promise Lance

Table 6. B: Follicle wave dynamics for cow ID 3713.

Wave #	Maximal Number of Antral Follicles	Baseline Number of Antral Follicles	Day of Emergence (d)	Day of Deviation (d)	Period of Dominance(d)	Classification	Dominant Follicle Growth (mm)			
							DF Dev	SUB Dev	DF Peak	SUB Peak
1	15	5.5	1	1	10	1	5.45	5.7	17.37	3.65
3	17	5	11	11	10	1	5.35	3.75	16.48	3.76
3	14	11	21	21	7	1	5.4	4.68	16.58	4.63
4	14.5	NA	NA	NA	NA	1	NA	NA	NA	NA

Table 6. C: Distribution of maximal number of antral follicles by size for cow ID 3713.

Wave #	Size of follicles (mm)					
	3 - 3.9	4 - 4.9	5 - 5.9	6 - 7.9	8 - 9.9	>10
1	11	3	3	4	2	1
2	8	4	4	1	1	2
3	8	2	4	5	3	2

Figure 7: Alterations in total numbers of antral follicles (≥ 3mm) during the first and ovulatory follicular waves of an estrous cycle and during the early stages of the first follicular wave of the next estrous cycle for cow ID 3713.

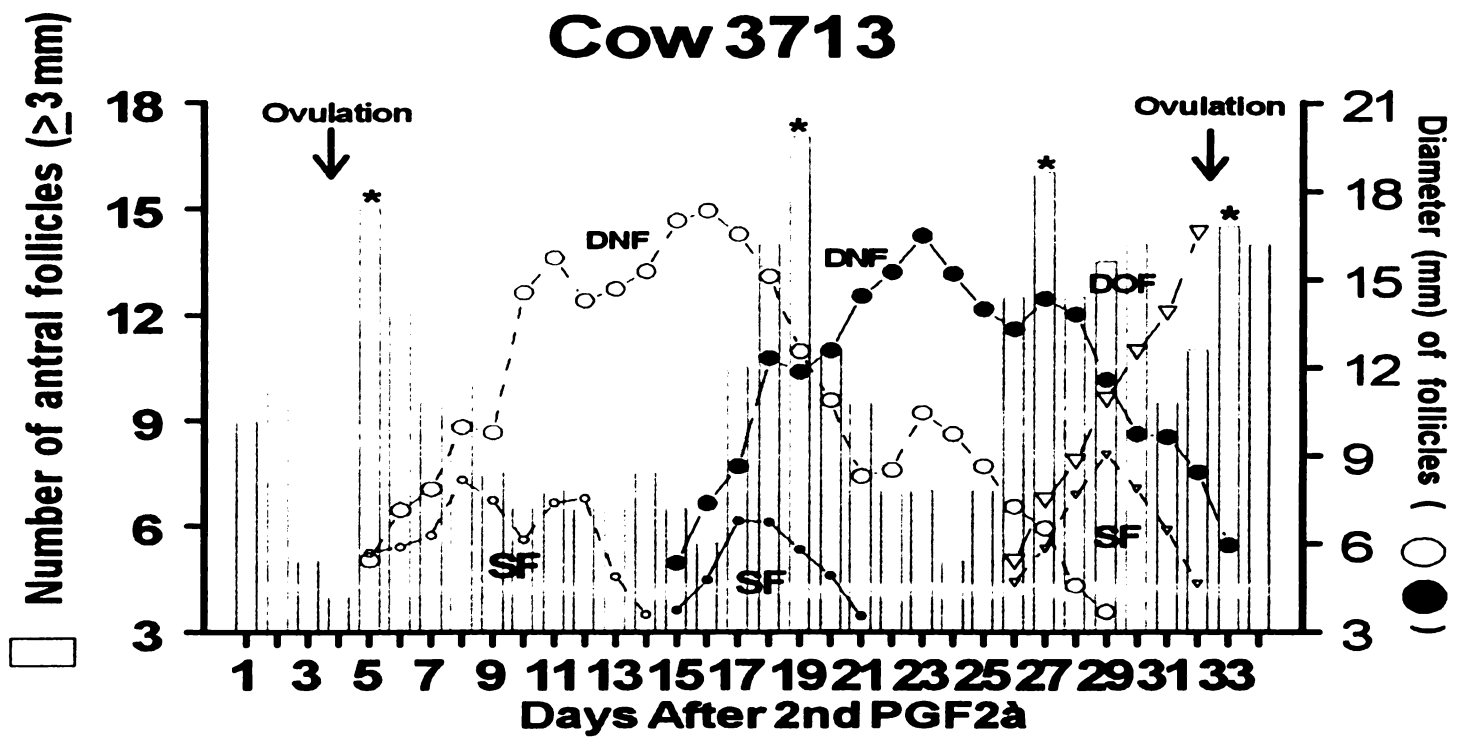


Table 7. A: Animal information for heifer 3879.

Animal ID	Age (yr.)	Milking Status	Sire	Dam	Dam's Sire
3879	1	NL	Ricecrest Brett	Michigan Marty Marti 3633	Ricecrest Marty

Table 7. B: Follicle wave dynamics for heifer ID 3879.

Wave #	Maximal Number of Antral Follicles	Baseline Number of Antral Follicles	Day of Emergence (d)	Day of Deviation (d)	Period of Dominance(d)	Classification	Milk Status	Dominant Follicle Growth			
								DF Dev	SUB Dev	DF Peak	SUB Peak
1	12	8.5	1	3	5	1	NL	5.75	5.23	14.85	3.64
2	12	6.5	10	10	10	1	NL	6.25	6.53	14.53	3.34
3	15	NA	NA	NA	NA	1	NL	NA	NA	NA	NA

Table 7. B: Distribution of maximal number of antral follicles by size for heifer ID 3879.

Wave #	Size of follicles (mm)					
	3 - 3.9	4 - 4.9	5 - 5.9	6 - 7.9	8 - 9.9	>10
1	12	4	4	3	2	2
2	7	1	3	4	4	2

Figure 8: Alterations in total numbers of antral follicles (≥ 3mm) during the first and ovulatory follicular waves of an estrous cycle and during the early stages of the first follicular wave of the next estrous cycle for heifer ID 3879.

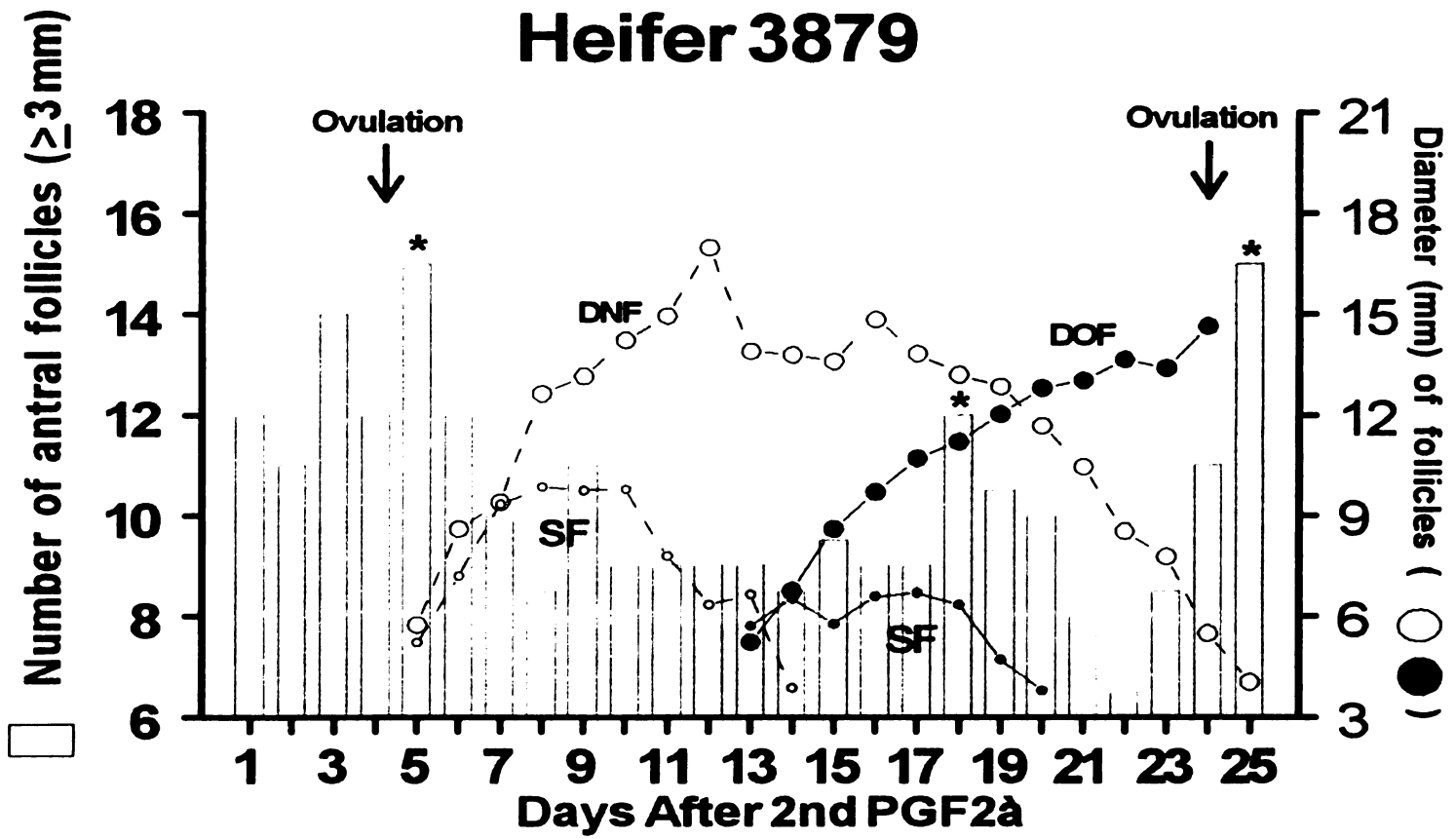


Table 8. A: Animal information for heifer ID 3880.

Animal ID	Age (yr.)	Milking Status	Sire	Dam	Dam's Sire
3880	1	NL	Ricecrest Emerson	NA	NA

Table 8. B: Follicle wave dynamics for heifer ID 3880.

Wave #	Maximal Number of Antral Follicles	Baseline Number of Antral Follicles	Day of Emergence (d)	Day of Deviation (d)	Period of Dominance(d)	Classification	Dominant Follicle Growth (mm)			
							DF Dev	SUB Dev	DF Peak	SUB Peak
1	12	6.5	1	2	8	1	7.75	7.03	14.53	3.8
2	13.5	8	10	14	8	1	7.13	7.38	13.95	5.98
3	14	NA	NA	NA	NA	1	NA	NA	NA	NA

Table 8. B: Distribution of maximal number of antral follicles by size for heifer ID 3880.

Wave #	Size of follicles (mm)					
	3 - 3.9	4 - 4.9	5 - 5.9	6 - 7.9	8 - 9.9	>10
1	14	4	3	4	2	1
2	10	2	3	2	2	2

Figure 9: Alterations in total numbers of antral follicles (≥ 3mm) during the first and ovulatory follicular waves of an estrous cycle and during the early stages of the first follicular wave of the next estrous cycle for heifer ID 3880.

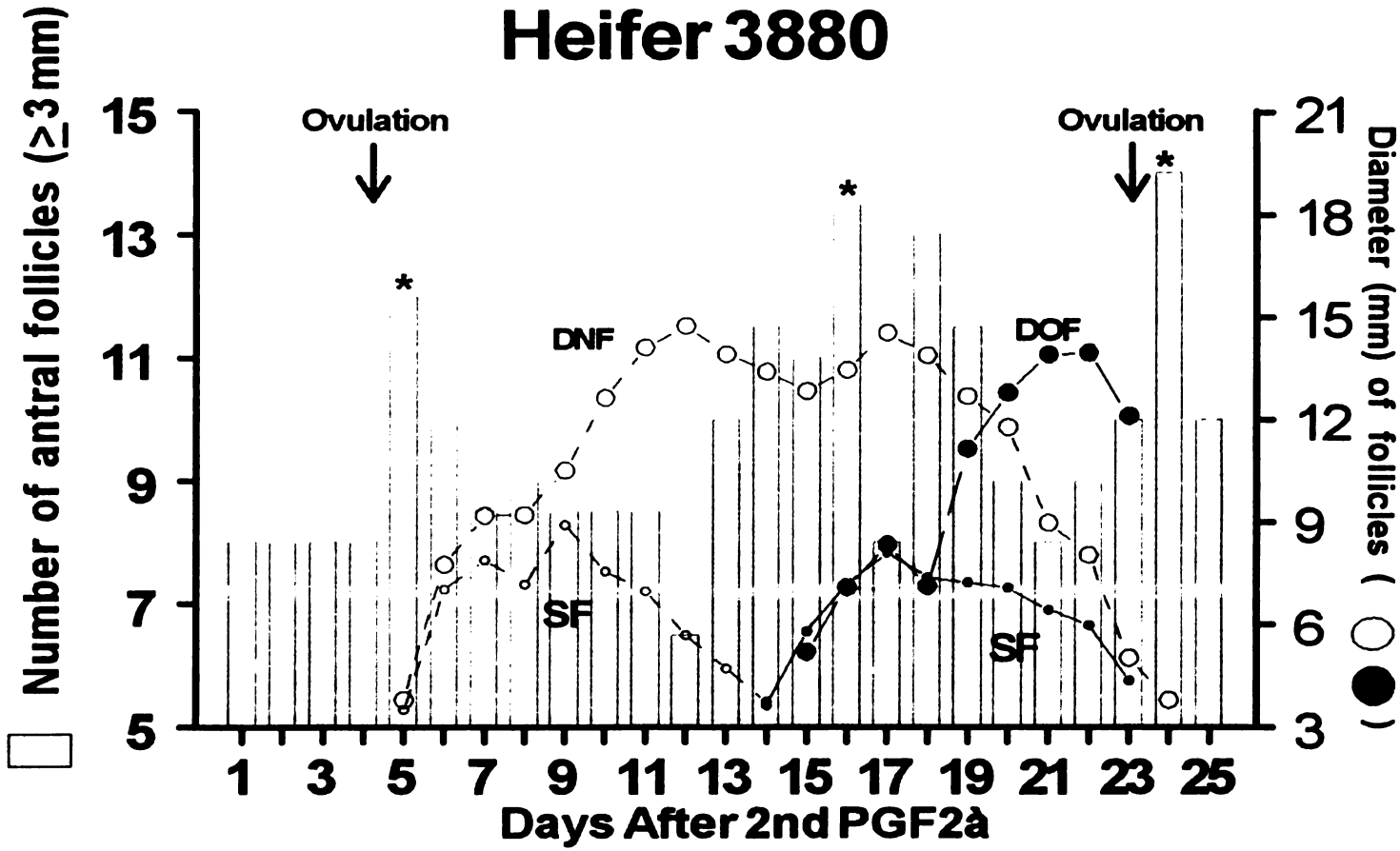


Table 9. A: Animal information for heifer 3887.

Animal ID	Age (yr.)	Milking Status	Sire	Dam	Dam's Sire
3887	1	NL	Peircefeild Chil Chamois	Michigan WCR Ullela 3636	Ladys-Manor Winchester

Table 9. B: Follicle wave dynamics for heifer ID 3887.

Wave #	Maximal Number of Antral Follicles	Baseline Number of Antral Follicles	Day of Emergence (d)	Day of Deviation (d)	Period of Dominance(d)	Classification	Dominant Follicle Growth (mm)			
							DF Dev	SUB Dev	DF Peak	SUB Peak
1	16.5	5.5	1	2	7	1	6.48	6.45	14.8	3.55
2	13.5	9	11	11	9	1	4.58	3.75	13.2	3.64
3	14.5	NA	NA	NA	NA	1	NA	NA	NA	NA

Table 9. C: Distribution of maximal number of antral follicles by size for heifer ID 3887.

Wave #	Size of follicles (mm)					
	3 - 3.9	4 - 4.9	5 - 5.9	6 - 7.9	8 - 9.9	>10
1	16	2	3	4	1	2
2	10	2	2	2	1	1

Figure 10: Alterations in total numbers of antral follicles (≥ 3mm) during the first and ovulatory follicular waves of an estrous cycle and during the early stages of the first follicular wave of the next estrous cycle for heifer ID 3887.

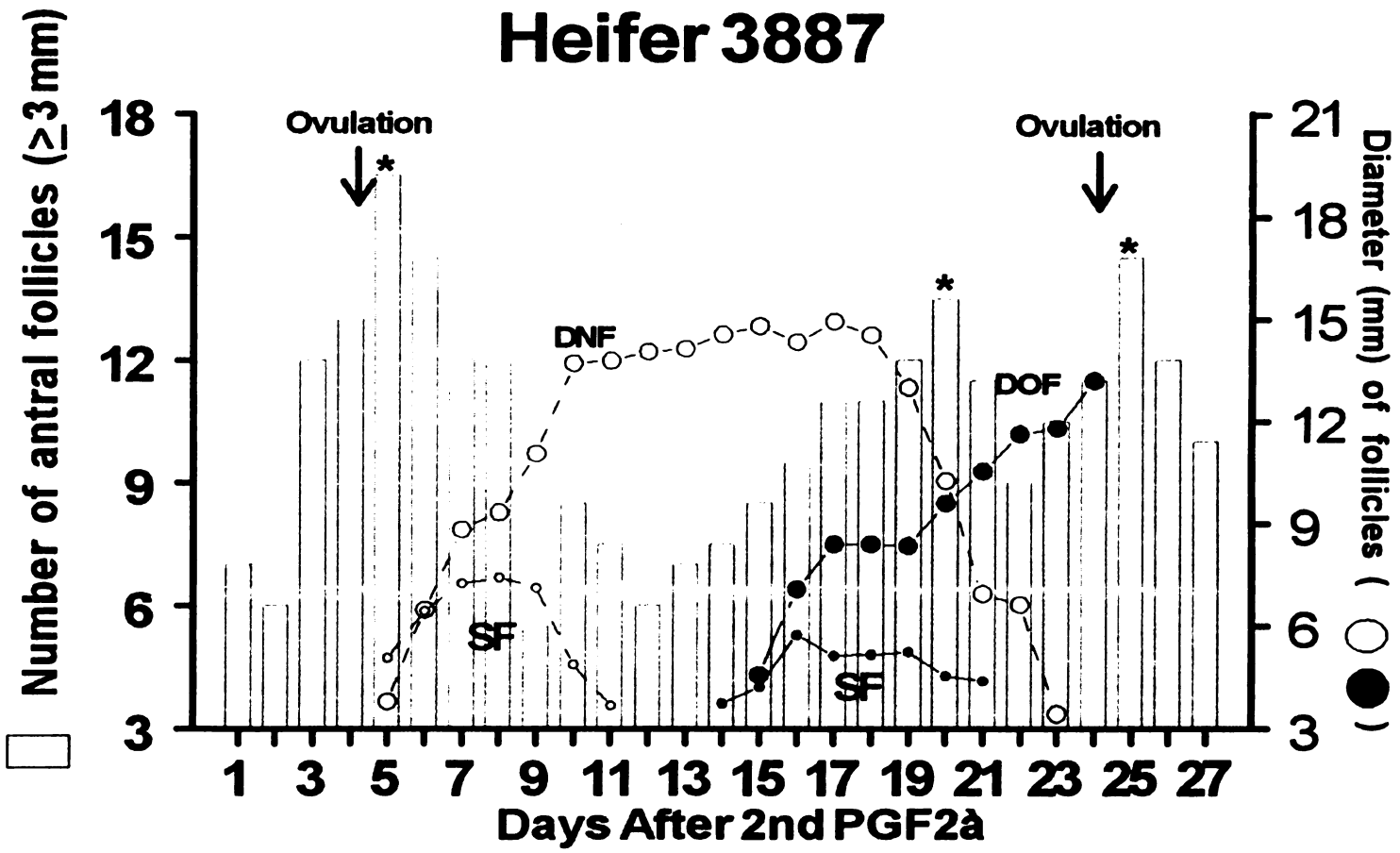


Table 10. A: Animal information of heifer ID 3889.

Animal ID	Age (yr.)	Milking Status	Sire	Dam	Dam's Sire
3889	1	NL	Corner-Pines Avalanche	Michigan Bran Charisse 3504	H-R-Z Tesk Brandon

Table 10. B: Follicle wave dynamics for heifer ID 3889.

Wave #	Maximal Number of Antral Follicles	Baseline Number of Antral Follicles	Day of Emergence (d)	Day of Deviation (d)	Period of Dominance(d)	Classification	Dominant Follicle Growth (mm)			
							DF Dev	SUB Dev	DF Peak	SUB Peak
1	12.5	4	1	1	6	1	5.65	5.55	15.3	4.78
2	12.5	2	8	8	12	1	4.2	3.75	13.78	3.65
3	11	NA	NA	NA	NA	1	NA	NA	NA	NA

Table 10. C: Distribution of maximal number of antral follicles by size for heifer ID 3889.

Wave #	Size of follicles (mm)					
	3 - 3.9	4 - 4.9	5 - 5.9	6 - 7.9	8 - 9.9	>10
1	10	2	2	2	1	1
2	11	2	2	1	1	1

Figure 11: Alterations in total numbers of antral follicles (≥ 3mm) during the first and ovulatory follicular waves of an estrous cycle and during the early stages of the first follicular wave of the next estrous cycle for heifer ID 3889.

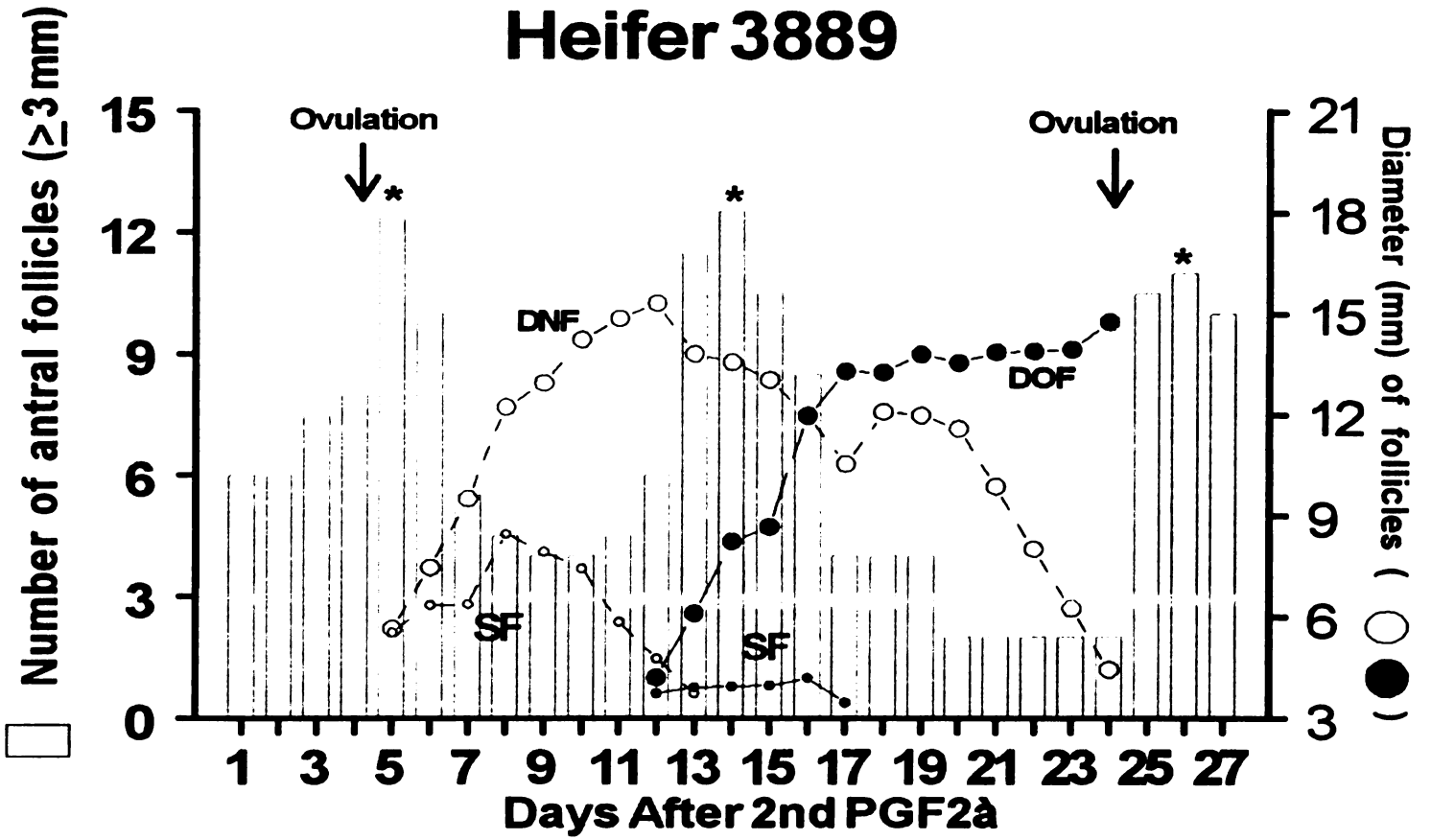


Table 11. A: Animal information for heifer ID 3896.

Animal ID	Age (yr.)	Milking Status	Sire	Dam	Dam's Sire
3896	1	NL	Dixie-Lee Aaron	Golden-View BW Gert 3420	Maizefeild Bellwood

Table 11. B: Follicle wave dynamics for heifer ID 3896.

Wave #	Maximal Number of Antral Follicles	Baseline Number of Antral Follicles	Day of Emergence (d)	Day of Deviation (d)	Period of Dominance(d)	Classification	Dominant Follicle Growth (mm)			
							DF Dev	SUB Dev	DF Peak	SUB Peak
1	16	7	1	2	5	1	5.3	5.95	15.2	3.94
2	15	5.5	9	9	6	1	3.97	3.75	13.43	3.54
3	12.5	6	17	20	4	1	7.45	7.45	15.52	3.67
4	15.5	NA	NA	NA	NA	1	NA	NA	NA	NA

Table 11. C: Distribution of maximal number of antral follicles by size for heifer ID 3896.

Wave #	Size of follicles (mm)					
	3 - 3.9	4 - 4.9	5 - 5.9	6 - 7.9	8 - 9.9	>10
1	16	5	4	4	1	1
2	13	4	3	4	1	2
3	14	4	3	4	1	1

Figure 12: Alterations in total numbers of antral follicles (≥ 3mm) during the first and ovulatory follicular waves of an estrous cycle and during the early stages of the first follicular wave of the next estrous cycle for heifer ID 3896.

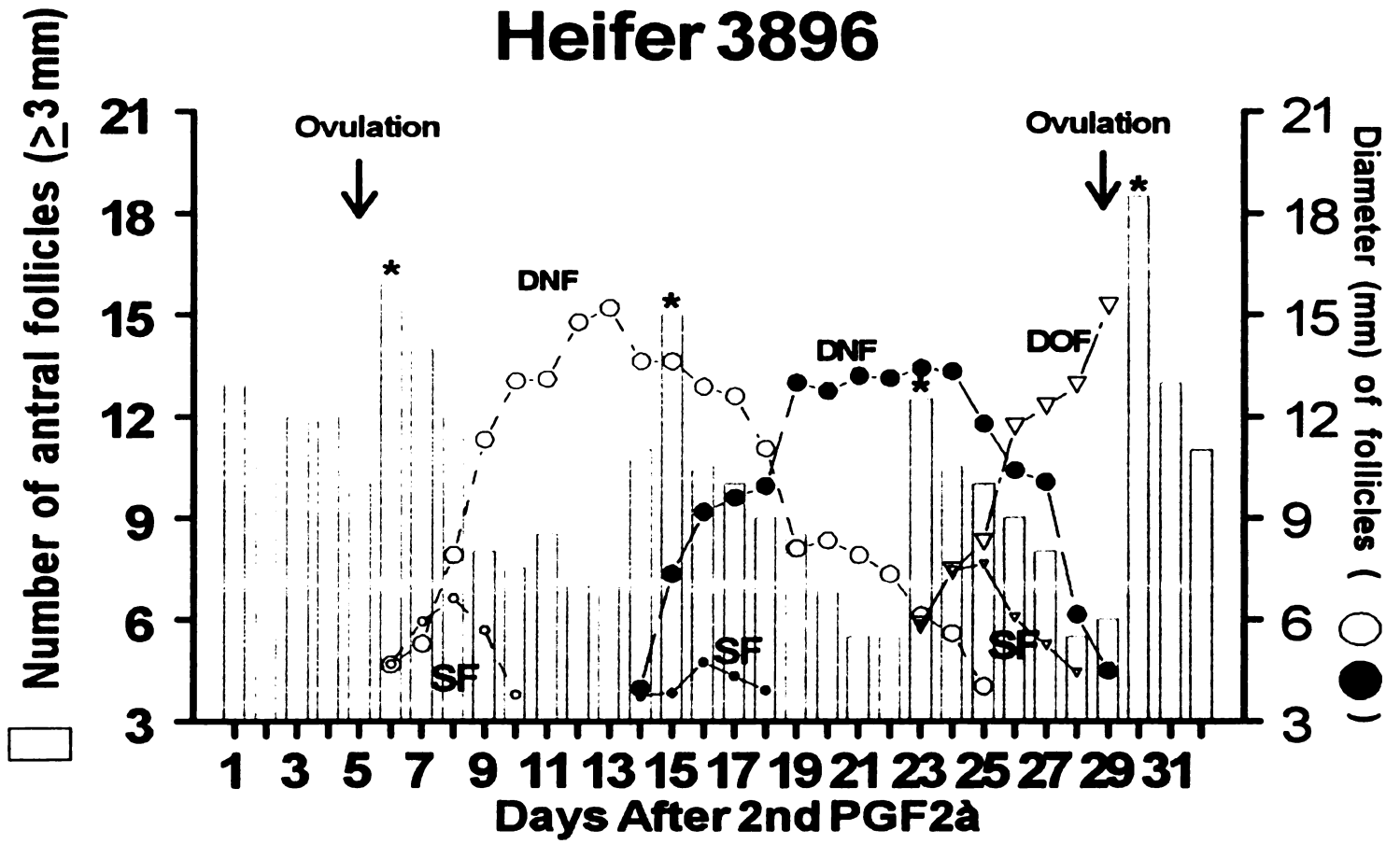


Table 12. A: Animal information for cow ID 3475.

Animal ID	Age (yr.)	Milking Status	Sire	Dam	Dam's Sire
3475	6	L	Zieland Zebo	Michigan Choice Daphne 2981	The Choice of Mark Adam

Table 12. A: Follicle wave dynamics for cow ID 3475.

Wave #	Maximal Number of Antral Follicles	Baseline Number of Antral Follicles	Day of Emergence (d)	Day of Deviation (d)	Period of Dominance(d)	Classification	Dominant Follicle Growth (mm)			
							DF Dev	SUB Dev	DF Peak	SUB Peak
1	20	7	2	3	5	2	7.25	4.88	11.58	3.86
2	19	9	9	11	NA	2	NA	NA	NA	NA

Table 12. B: Distribution of maximal number of antral follicles by size for cow ID 3475.

Wave #	Size of follicles (mm)					
	3 - 3.9	4 - 4.9	5 - 5.9	6 - 7.9	8 - 9.9	>10
1	16	3	2	4	3	1
2	17	6	3	3	2	2

Figure 13: Alterations in total numbers of antral follicles (≥ 3mm) during the first and ovulatory follicular waves of an estrous cycle and during the early stages of the first follicular wave of the next estrous cycle for cow ID 3475.

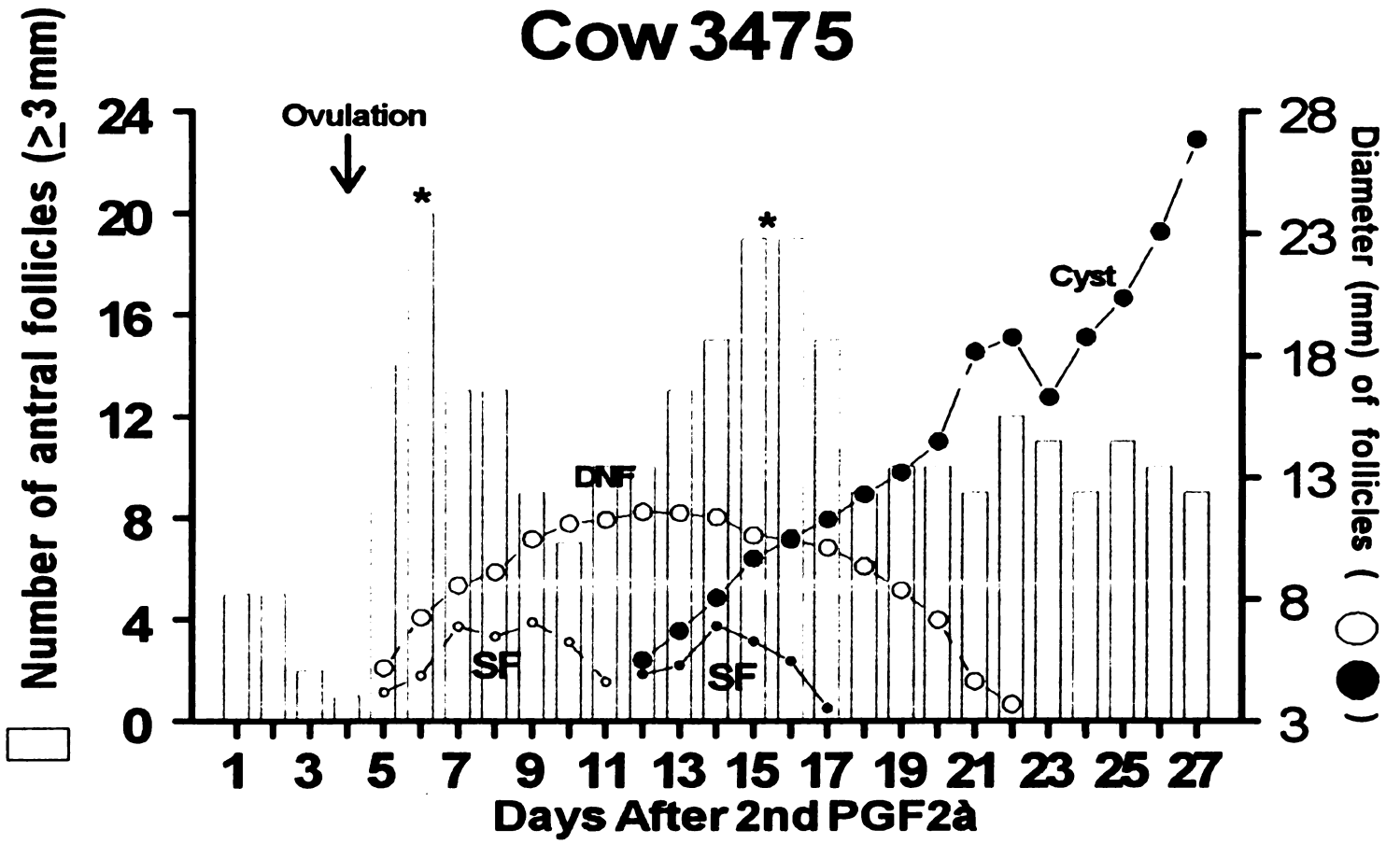


Table 13. A: Animal information for cow ID 3507.

Animal ID	Age (yr.)	Milking Status	Sire	Dam	Dam's Sire
3507	5	NL	Diamond-W Wind Kaddy	Kellogg-MSU C Telmo 3158	Vo-Sin Rotate Classic

Table 13. B: Follicle wave dynamics for cow ID 3507.

Wave #	Maximal Number of Antral Follicles	Baseline Number of Antral Follicles	Day of Emergence (d)	Day of Deviation (d)	Period of Dominance(d)	Classification	Dominant Follicle Growth (mm)			
							DF Dev	SUB Dev	DF Peak	SUB Peak
1	20	6	1	1	10	2	3.35	3.68	15.7	3.67
2	20	7	11	13	10	2	6.28	5.33	14.28	3.63
3	20	NA	NA	NA	NA	2	NA	NA	NA	NA

Table 13. C: Distribution of maximal number of antral follicles by size for cow ID 3507.

Wave #	Size of follicles (mm)					
	3 - 3.9	4 - 4.9	5 - 5.9	6 - 7.9	8 - 9.9	>10
1	19	7	4	2	1	1
2	15	3	2	3	1	1

Figure 14: Alterations in total numbers of antral follicles (≥ 3mm) during the first and ovulatory follicular waves of an estrous cycle and during the early stages of the first follicular wave of the next estrous cycle for cow ID 3507.

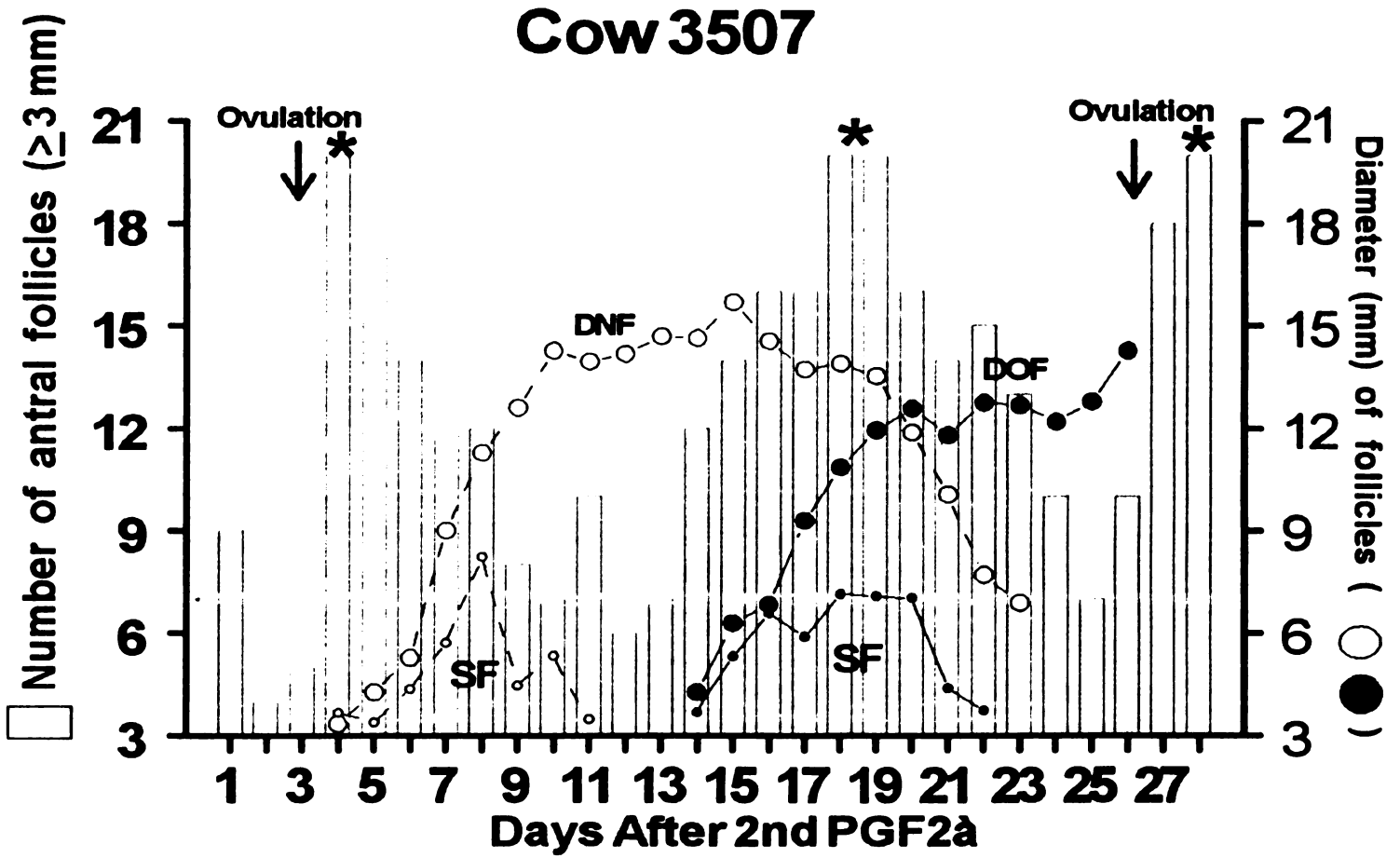


Table 14. A: Animal information for cow ID 3534.

Animal ID	Age (yr.)	Milking Status	Sire	Dam	Dam's Sire
3534	5	NL	End-Road Leadman Barlo	Michigan Nick Niki 3229	United Nick

Table 14. B: Follicle wave dynamics for cow ID 3534.

Wave #	Maximal Number of Antral Follicles	Baseline Number of Antral Follicles	Day of Emergence (d)	Day of Deviation (d)	Period of Dominance(d)	Classification	Dominant Follicle Growth (mm)			
							DF Dev	SUB Dev	DF Peak	SUB Peak
1	18	5	1	5	7	2	11.5	10.5	14.75	3.45
2	16	5	12	12	12	2	4	3.7	14.35	3.67
3	17.5	NA	NA	NA	NA	2	NA	NA	NA	NA

Table 14. C: Distribution of maximal number of antral follicles by size for cow ID 3534.

	Size of follicles (mm)					
Wave #	3 - 3.9	4 - 4.9	5 - 5.9	6 - 7.9	8 - 9.9	>10
1	15	4	3	3	2	2
2	10	4	3	3	1	2

Figure 15: Alterations in total numbers of antral follicles (≥ 3mm) during the first and ovulatory follicular waves of an estrous cycle and during the early stages of the first follicular wave of the next estrous cycle for cow ID 3534.

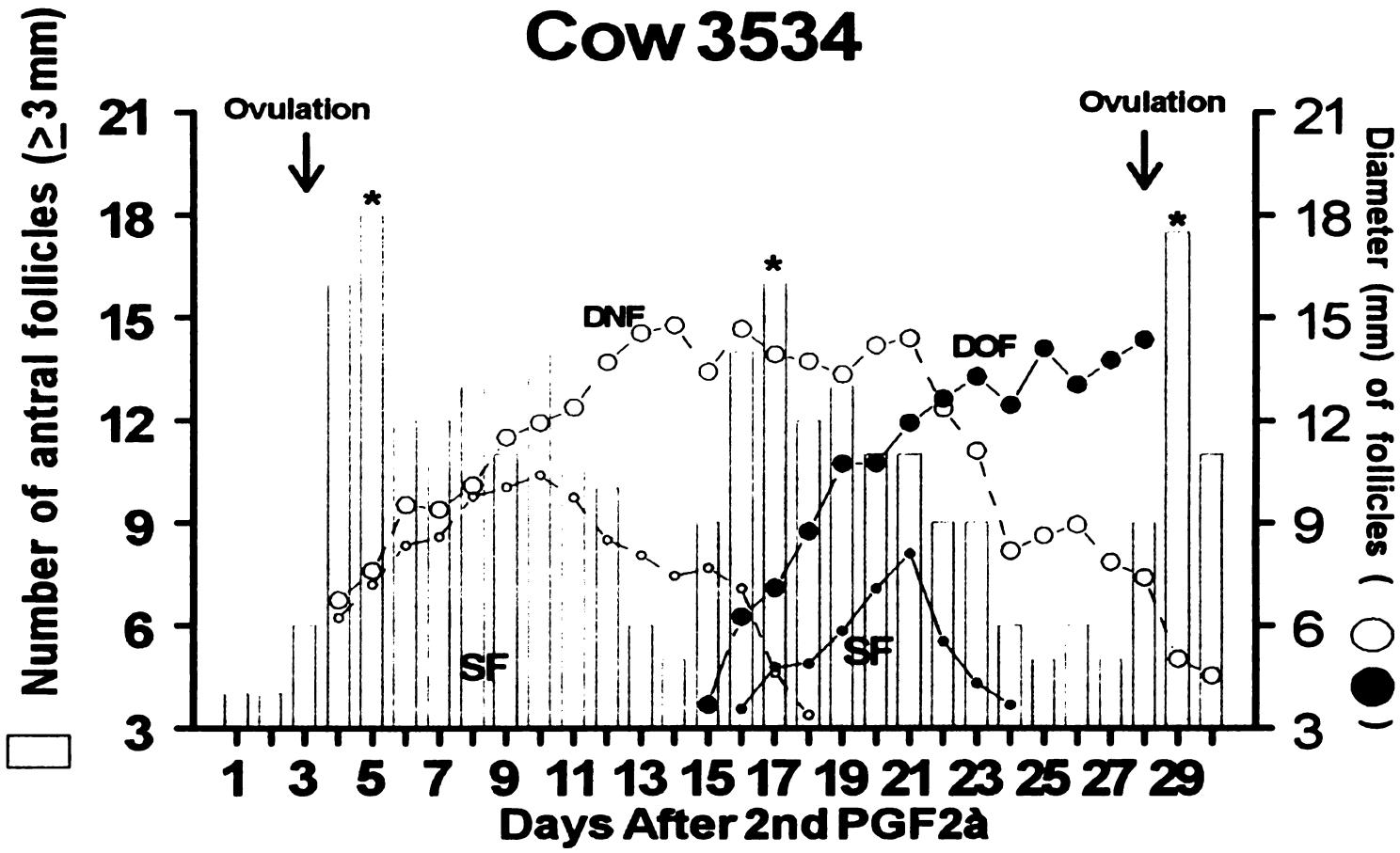


Table 15. A: Animal information for cow ID 3695.

Animal ID	Age (yr.)	Milking Status	Sire	Dam	Dam's Sire
3695	4	L	WA-Del Bubba	Michigan Mascot Cream 3481	Singing-Brook N-B Mascot

Table 15. B: Follicle wave dynamics for cow ID 3695.

Wave #	Maximal Number of Antral Follicles	Baseline Number of Antral Follicles	Day of Emergence (d)	Day of Deviation (d)	Period of Dominance(d)	Classification	Dominant Follicle Growth (mm)			
							DF Dev	SUB Dev	DF Peak	SUB Peak
1	18	10	1	1	9	2	5.5	5.43	17.1	3.65
2	18	8.5	10	10	7	2	5.45	5.68	13.65	3.7
3	17	14	17	18	3	2	6.73	7.45	13.5	6.25
4	19.5	NA	NA	NA	NA	2	NA	NA	NA	NA

Table 15. C: Distribution of maximal number of antral follicles by size for cow ID 3695.

Wave #	Size of follicles (mm)					
	3 - 3.9	4 - 4.9	5 - 5.9	6 - 7.9	8 - 9.9	>10
1	14	3	4	5	1	1
2	16	4	6	2	1	2
3	11	4	4	4	1	2

Figure 16: Alterations in total numbers of antral follicles (≥ 3mm) during the first and ovulatory follicular waves of an estrous cycle and during the early stages of the first follicular wave of the next estrous cycle for cow ID 3695.

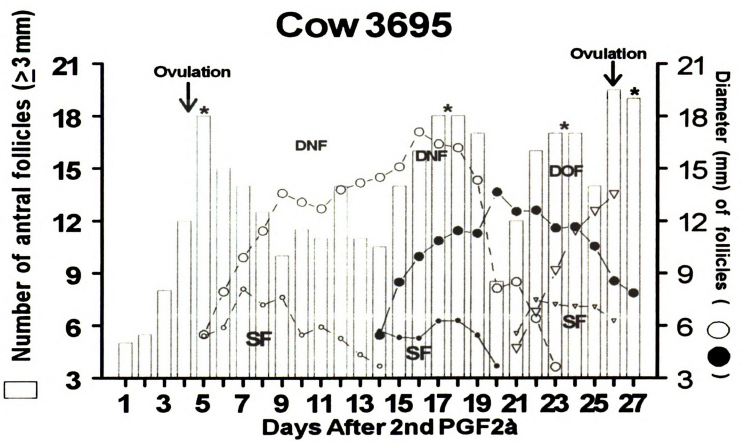


Table 16. A: Animal information for cow ID 3704.

Animal ID	Age (yr.)	Milking Status	Sire	Dam	Dam's Sire
3704	4	L	Ricecrest Tesk Terry	Michigan Aristds Yo-Yo 3353	Howcrest Aristides

Table 16. B: Follicle wave dynamics for cow ID 3704.

Wave #	Maximal Number of Antral Follicles	Baseline Number of Antral Follicles	Day of Emergence (d)	Day of Deviation (d)	Period of Dominance(d)	Classification	Dominant Follicle Growth (mm)			
							DF Dev	SUB Dev	DF Peak	SUB Peak
1	16.5	9	1	3	8	2	6.62	9.075	16.23	6.65
2	20	12	13	13	10	2	6.28	5.3	17.8	3.5
3	18	NA	NA	NA	NA	2	NA	NA	NA	NA

Table 16. C: Distribution of maximal number of antral follicles by size for cow ID 3704.

Wave #	Size of follicles (mm)					
	3 - 3.9	4 - 4.9	5 - 5.9	6 - 7.9	8 - 9.9	>10
1	14	4	3	2	3	2
2	14	6	5	4	1	2

Figure 17: Alterations in total numbers of antral follicles (≥ 3mm) during the first and ovulatory follicular waves of an estrous cycle and during the early stages of the first follicular wave of the next estrous cycle for cow ID 3704.

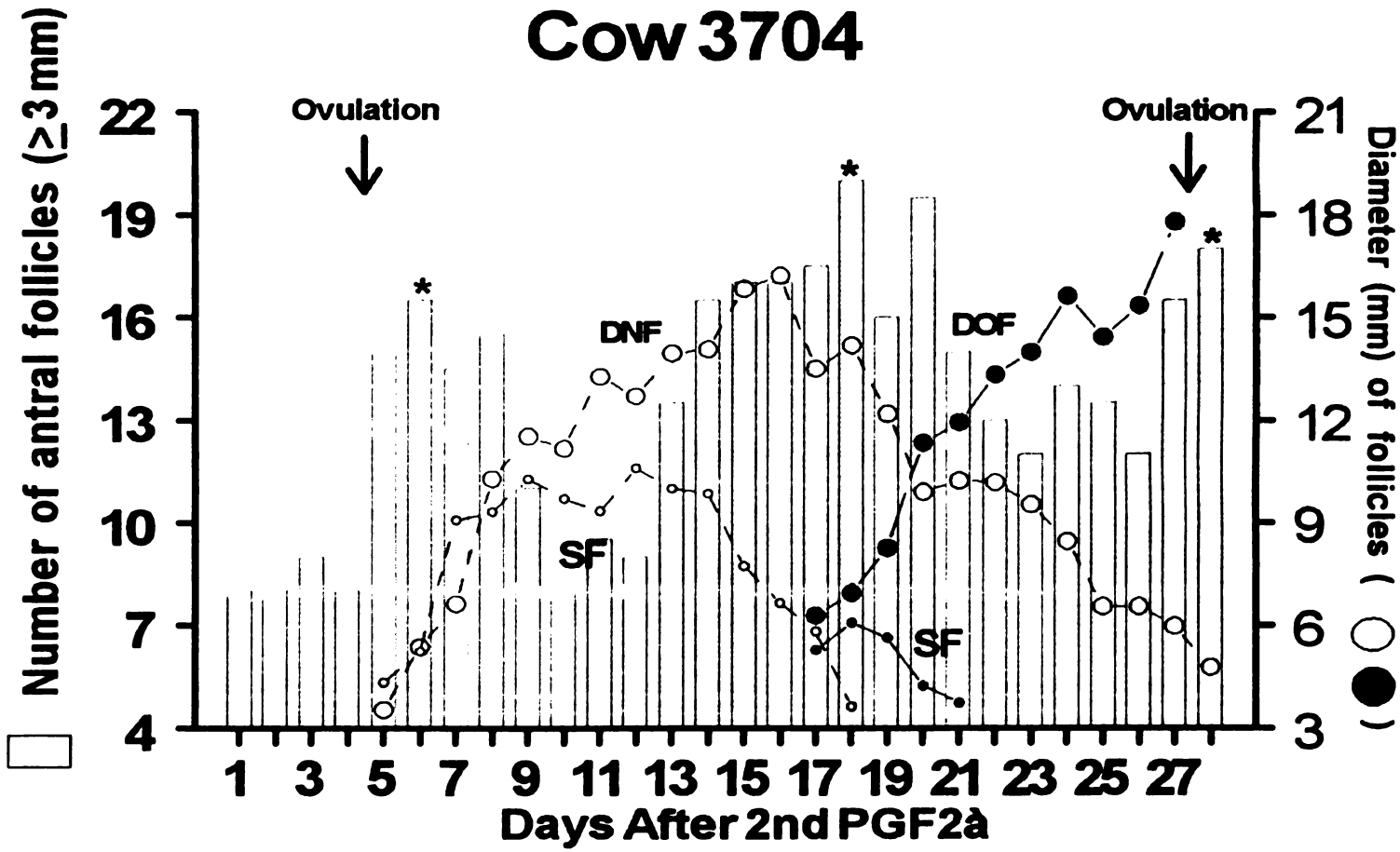


Table 17. A: Animal information for heifer ID 3894.

Animal ID	Age (yr.)	Milking Status	Sire	Dam	Dam's Sire
3894	1	NL	Ricecrest Brett	Michigan Duster Jill 3609	Penn-Col Duster

Table 17. B: Follicle wave dynamics for heifer ID 3894.

Wave #	Maximal Number of Antral Follicles	Baseline Number of Antral Follicles	Day of Emergence (d)	Day of Deviation (d)	Period of Dominance(d)	Classification	Dominant Follicle Growth (mm)			
							DF Dev	SUB Dev	DF Peak	SUB Peak
1	19.5	3.5	1	3	5	2	6.65	9.05	15.775	3.64
2	18	4	9	10	10	2	5.2	3.8	16.56	3.71
3	18	NA	NA	NA	NA	2	NA	NA	NA	NA

Table 17. C: Distribution of maximal number of antral follicles by size for heifer ID 3894.

Wave #	Size of follicles (mm)					
	3 - 3.9	4 - 4.9	5 - 5.9	6 - 7.9	8 - 9.9	>10
1	14	3	4	3	2	1
2	16	4	4	2	2	2

Figure 18: Alterations in total numbers of antral follicles (≥ 3mm) during the first and ovulatory follicular waves of an estrous cycle and during the early stages of the first follicular wave of the next estrous cycle for heifer ID 3894.

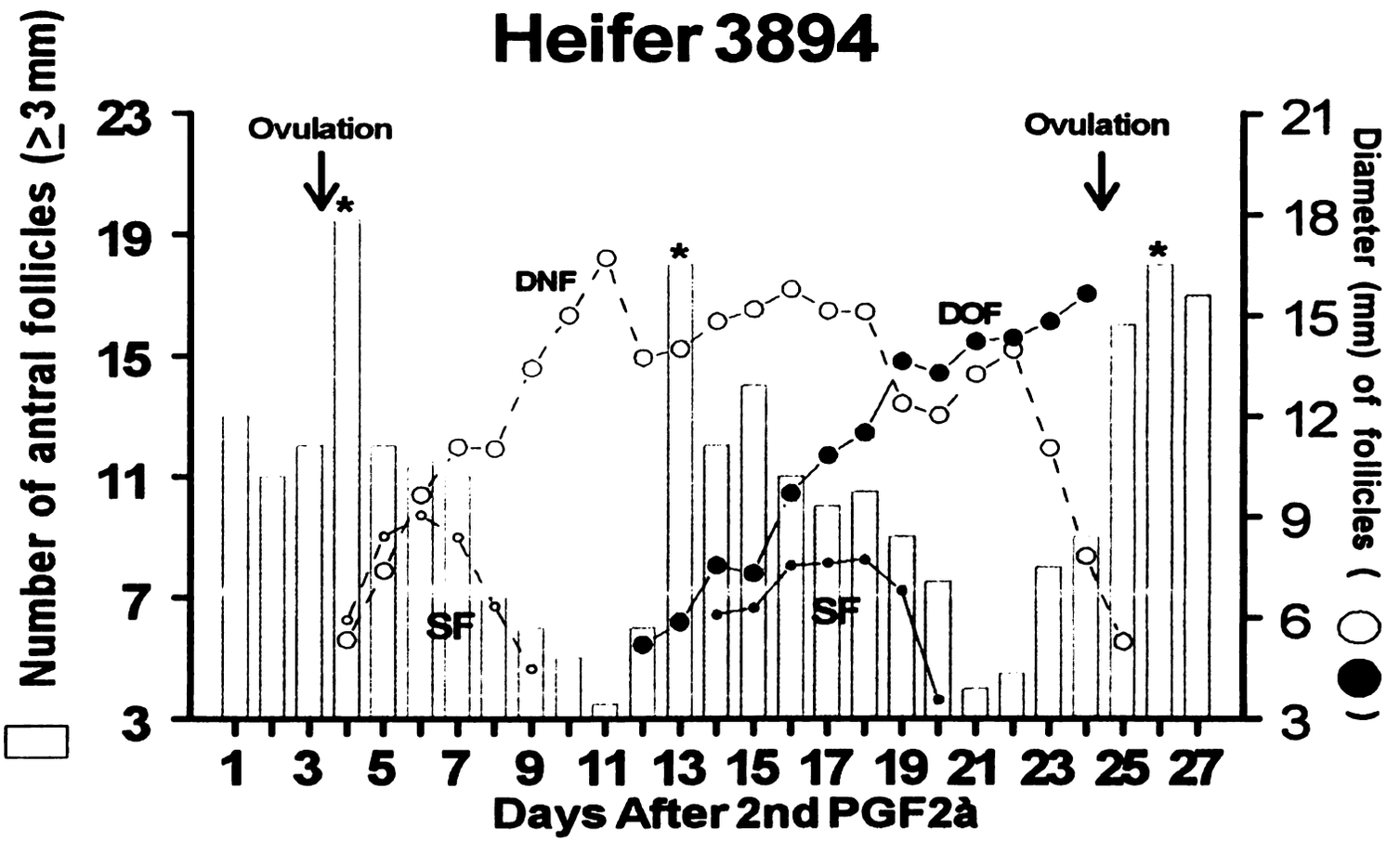


Table 18. A: Animal information for heifer ID 3901.

Animal ID	Age (yr.)	Milking Status	Sire	Dam	Dam's Sire
3901	1	NL	Lutz-Medows MB Mozer	D-P-M Patn Linda 3444	Brabant Star Patron

Table 18. B: Follicle wave dynamics for heifer ID 3901.

Wave #	Maximal Number of Antral Follicles	Baseline Number of Antral Follicles	Day of Emergence (d)	Day of Deviation (d)	Period of Dominance(d)	Classification	Dominant Follicle Growth (mm)			
							DF Dev	SUB Dev	DF Peak	SUB Peak
1	16	5.5	1	2	6	2	4.83	6.1	14.63	3.41
3	18	6.5	16	16	5	2	5.28	5.13	13.98	3.31
3	14	8.5	9	9	5	2	4.7	5.35	13.15	3.8
4	15.5	NA	NA	NA	NA	2	NA	NA	NA	NA

Table 18. C: Distribution of maximal number of antral follicles by size for heifer ID 3901.

Wave #	Size of follicles (mm)					
	3 - 3.9	4 - 4.9	5 - 5.9	6 - 7.9	8 - 9.9	>10
1	13	3	3	2	1	1
2	12	3	3	2	1	2
3	15	3	3	4	2	1

Figure 19: Alterations in total numbers of antral follicles (≥ 3mm) during the first and ovulatory follicular waves of an estrous cycle and during the early stages of the first follicular wave of the next estrous cycle for heifer ID 3901.

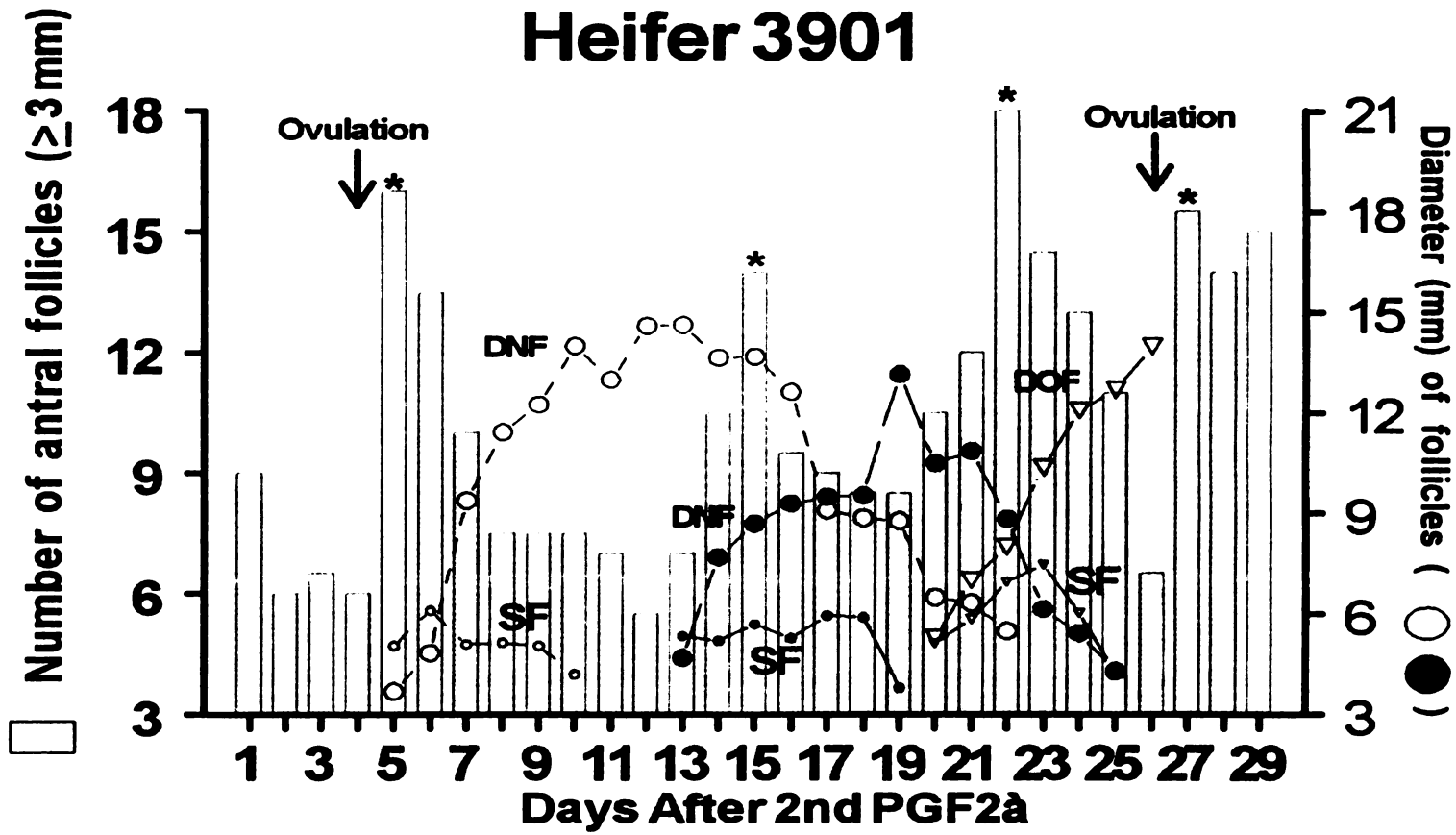


Table 19. A: Animal information for heifer ID 3903.

Animal ID	Age (yr.)	Milking Status	Sire	Dam	Dam's Sire
3903	1	NL	Dixie-Lee Aaron	Michigan Merrl Marylee 3212	Londondale Merrill

Table 19. B: Follicle wave dynamics for heifer ID 3903.

Wave #	Maximal Number of Antral Follicles	Baseline Number of Antral Follicles	Day of Emergence (d)	Day of Deviation (d)	Period of Dominance(d)	Classification	Dominant Follicle Growth (mm)			
							DF Dev	SUB Dev	DF Peak	SUB Peak
1	17	5	1	2	7	2	4.625	4.475	15.425	3.62
2	17	7.5	11	11	13	2	4.525	6.075	17.15	3.21
3	14.5	NA	NA	NA	NA	2	NA	NA	NA	NA

Table 19. C: Distribution of maximal number of antral follicles by size for heifer ID 3903.

Wave #	Size of follicles (mm)					
	3 - 3.9	4 - 4.9	5 - 5.9	6 - 7.9	8 - 9.9	>10
1	14	6	5	3	2	1
2	17	4	4	3	2	2

Figure 20: Alterations in total numbers of antral follicles (≥ 3mm) during the first and ovulatory follicular waves of an estrous cycle and during the early stages of the first follicular wave of the next estrous cycle for heifer ID 3903.

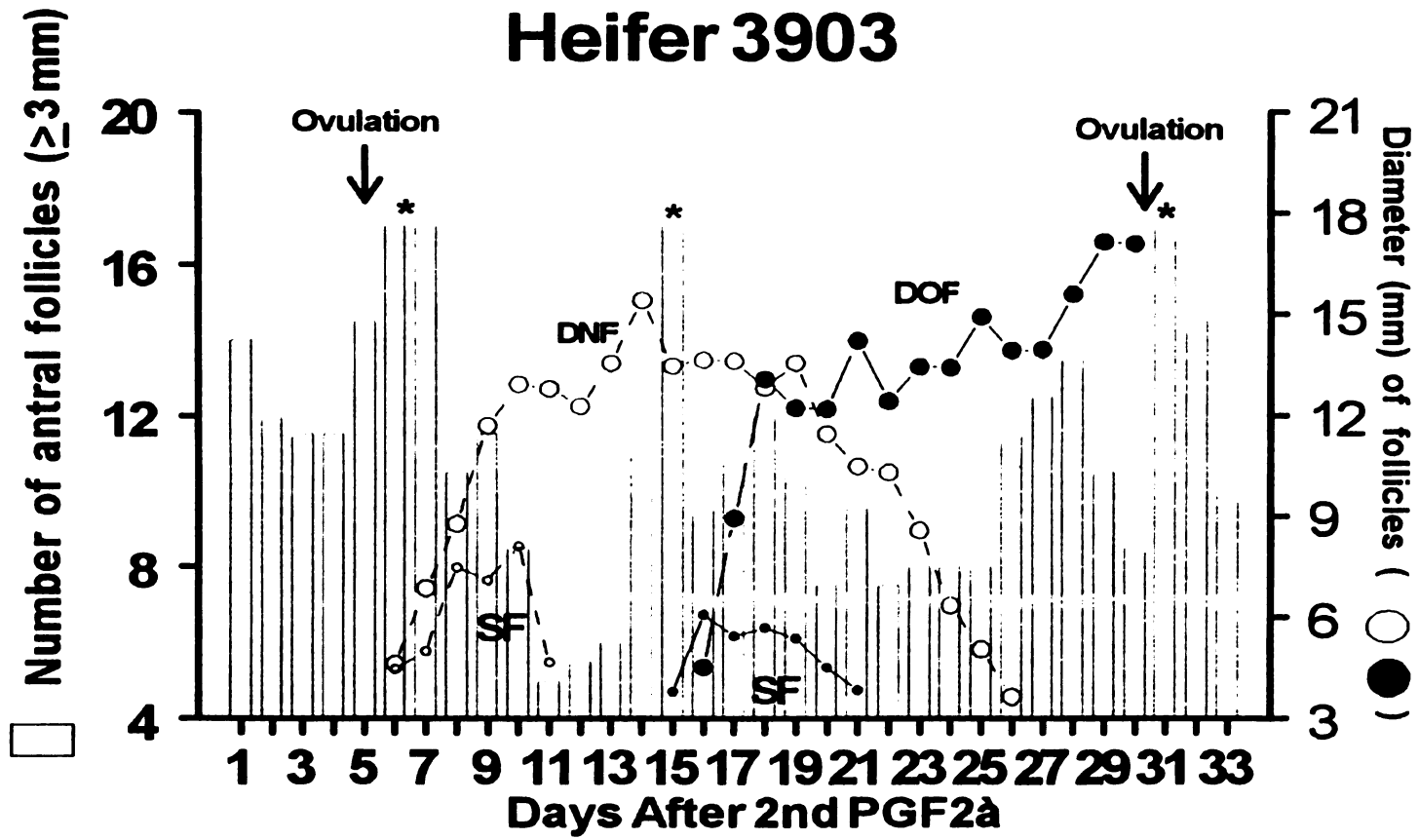


Table 20. A: Animal information for cow ID 3160.

Animal ID	Age (yr.)	Milking Status	Sire	Dam	Dam's Sire
3160	7	NL	The Choice of Mark Adam	Kellogg-Msu Blackstar Peony	To-Mar Blackstar

Table 20. B: Follicle wave dynamics for cow ID 3160.

Wave #	Maximal Number of Antral Follicles	Baseline Number of Antral Follicles	Day of Emergence (d)	Day of Deviation (d)	Period of Dominance(d)	Classification	Dominant Follicle Growth (mm)			
							DF Dev	SUB Dev	DF Peak	SUB Peak
1	23	12	1	1	10	3	7.45	4.9	18	4.7
2	23	11	11	12	3	3	11.83	6.18	16.05	3.3
3	25	16	14	14	13	3	10.65	3.7	14.6	3.75
4	26	NA	NA	NA	NA	3	NA	NA	NA	NA

Table 20. C: Distribution of maximal number of antral follicles by size for cow ID 3160.

Wave #	Size of follicles (mm)					
	3 - 3.9	4 - 4.9	5 - 5.9	6 - 7.9	8 - 9.9	>10
1	20	7	5	4	2	1
2	19	8	4	4	1	1

Figure 21: Alterations in total numbers of antral follicles (≥ 3mm) during the first and ovulatory follicular waves of an estrous cycle and during the early stages of the first follicular wave of the next estrous cycle for cow ID 3160.

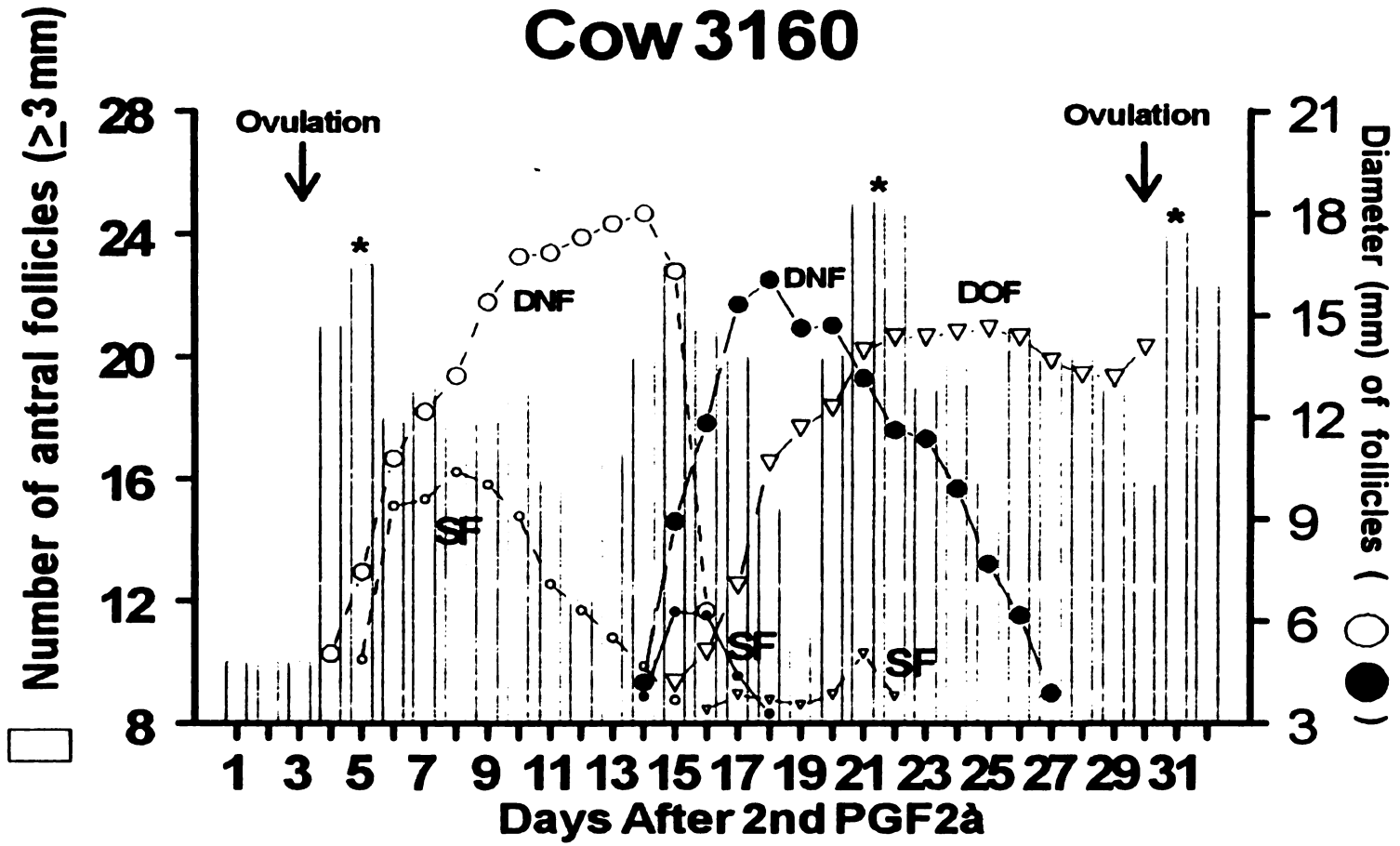


Table 21. A: Animal information for cow ID 3459.

Animal ID	Age (yr.)	Milking Status	Sire	Dam	Dam's Sire
3459	5	L	Windcrest Leadman Judge	Michigan Hunter Prize 3256	Ameldin II Pontiac Hunter

Table 21. B: Follicle wave dynamics for cow ID 3459.

Wave #	Maximal Number of Antral Follicles	Baseline Number of Antral Follicles	Day of Emergence (d)	Day of Deviation (d)	Period of Dominance(d)	Classification	Dominant Follicle Growth (mm)			
							DF Dev	SUB Dev	DF Peak	SUB Peak
1	24	10	1	1	7	3	9.33	4.78	16.45	3.25
2	20	9	8	14	7	3	11.6	10.08	16.63	8.93
3	21	NA	NA	NA	NA	3	NA	NA	NA	NA

Table 21. C: Distribution of maximal number of antral follicles by size for cow ID 3459.

Wave #	Size of follicles (mm)					
	3 - 3.9	4 - 4.9	5 - 5.9	6 - 7.9	8 - 9.9	>10
1	20	2	2	2	1	1
2	14	7	3	2	2	2

Figure 22: Alterations in total numbers of antral follicles (≥ 3mm) during the first and ovulatory follicular waves of an estrous cycle and during the early stages of the first follicular wave of the next estrous cycle for cow ID 3459.

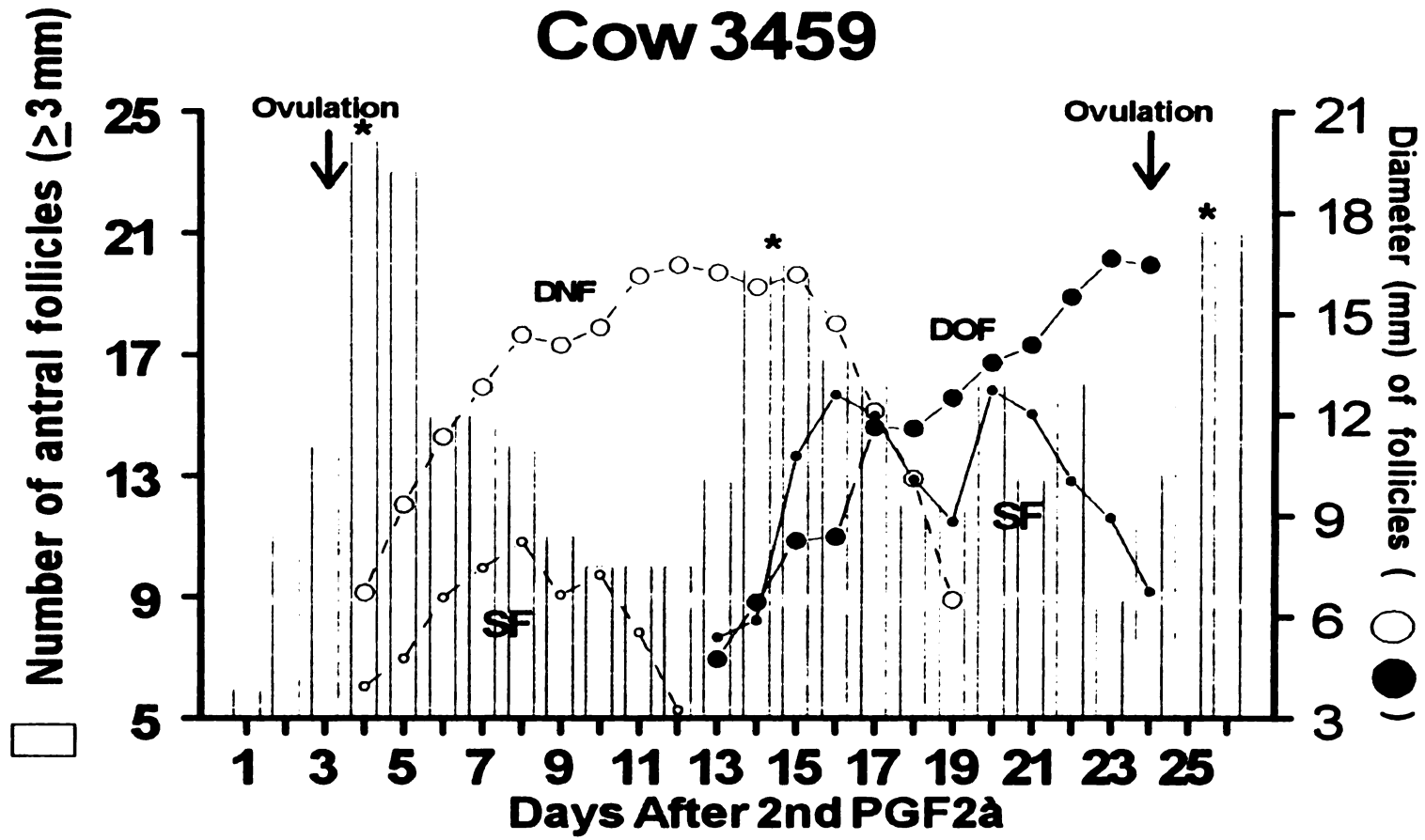


Table 22. A: Animal information for cow ID 3533.

Animal ID	Age (yr.)	Milking Status	Sire	Dam	Dam's Sire
3533	4	L	Shen-Val NV LM Formation	Michigan Elvin Arbor 3236	Gen-Ace Petrum LD Elvin

Table 22. B: Follicle wave dynamics for cow ID 3533.

Wave #	Maximal Number of Antral Follicles	Baseline Number of Antral Follicles	Day of Emergence (d)	Day of Deviation (d)	Period of Dominance(d)	Classification	Dominant Follicle Growth (mm)			
							DF Dev	SUB Dev	DF Peak	SUB Peak
1	20	14.5	1	5	7	3	13.65	7.6	17.55	3.67
2	24	13	11	16	8	3	5.88	5.9	19	3.65
3	24	NA	NA	NA	NA	3	NA	NA	NA	NA

Table 22. C: Distribution of maximal number of antral follicles by size for cow ID 3533.

Wave #	Size of follicles (mm)					
	3 - 3.9	4 - 4.9	5 - 5.9	6 - 7.9	8 - 9.9	>10
1	17	3	3	3	2	3
2	21	4	3	2	1	2

Figure 23: Alterations in total numbers of antral follicles (≥ 3mm) during the first and ovulatory follicular waves of an estrous cycle and during the early stages of the first follicular wave of the next estrous cycle for cow ID 3533.

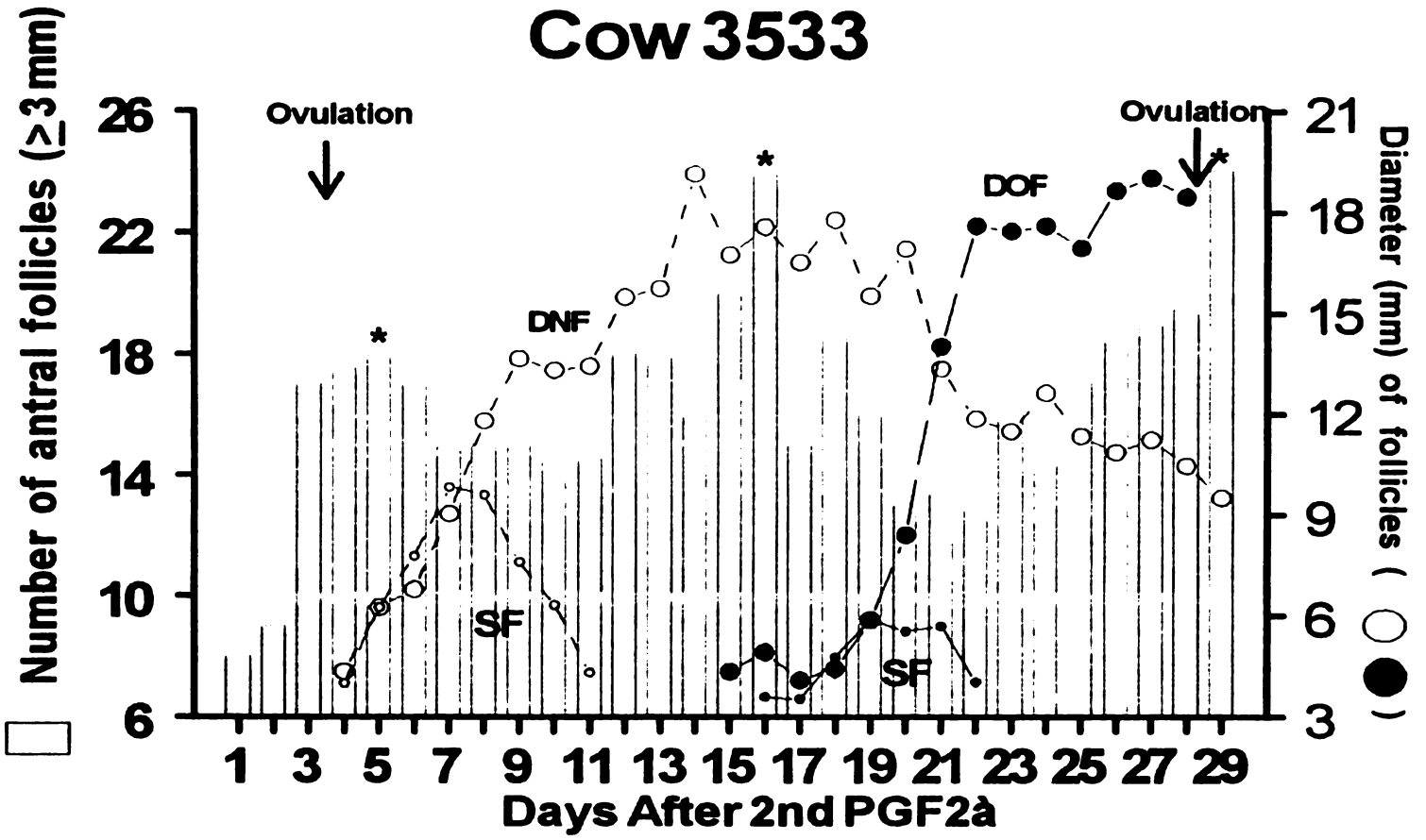


Table 23. A: Animal information for cow ID 3785.

Animal ID	Age (yr.)	Milking Status	Sire	Dam	Dam's Sire
3785	4	NL	NA	NA	NA

Table 23. B: Follicle wave dynamics for cow ID 3785.

Wave #	Maximal Number of Antral Follicles	Baseline Number of Antral Follicles	Day of Emergence (d)	Day of Deviation (d)	Period of Dominance(d)	Classification	Dominant Follicle Growth (mm)			
							DF Dev	SUB Dev	DF Peak	SUB Peak
1	22	9.5	1	3	5	3	6.78	7.73	14.5	3.55
2	19.5	10.5	9	9	10	3	5.28	4.98	15.56	3.89
3	19.5	NA	NA	NA	NA	3	NA	NA	NA	NA

Table 23. C: Distribution of maximal number of antral follicles by size for cow ID 3785.

Wave #	Size of follicles (mm)					
	3 - 3.9	4 - 4.9	5 - 5.9	6 - 7.9	8 - 9.9	>10
1	16	9	4	4	2	1
2	15	5	2	3	2	1

Figure 24: Alterations in total numbers of antral follicles (≥ 3mm) during the first and ovulatory follicular waves of an estrous cycle and during the early stages of the first follicular wave of the next estrous cycle for cow ID 3785.

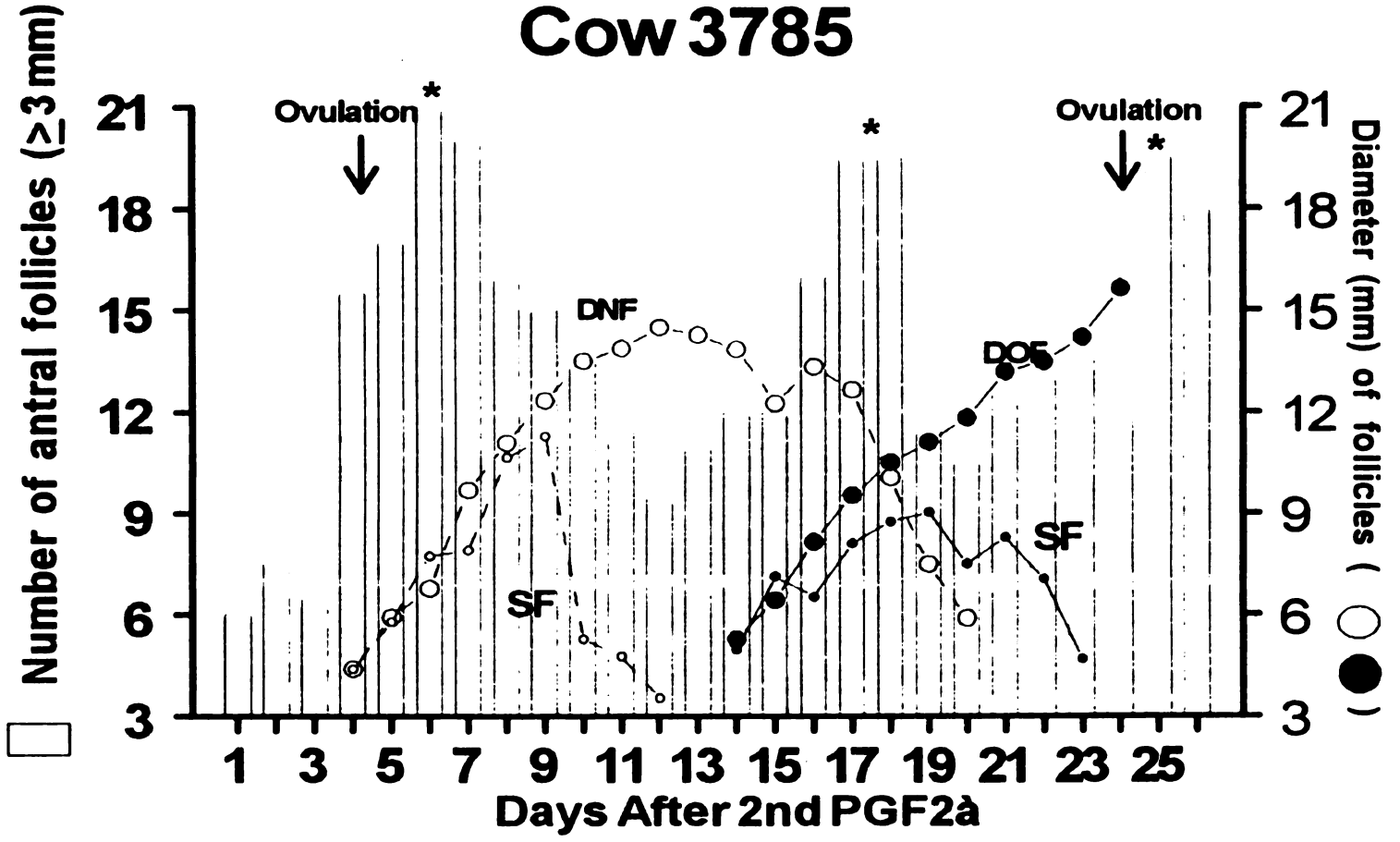


Table 24. A: Animal information for heifer 3892.

Animal ID	Age (yr.)	Milking Status	Sire	Dam	Dam's Sire
3892	1	NL	Michigan MR Olympus 9653	Michigan GEF Crissy 3379	Mar-Bill Command Geoffry

Table 24. B: Follicle wave dynamics for heifer ID 3982.

Wave #	Maximal Number of Antral Follicles	Baseline Number of Antral Follicles	Day of Emergence (d)	Day of Deviation (d)	Period of Dominance(d)	Classification	Dominant Follicle Growth (mm)			
							DF Dev	SUB Dev	DF Peak	SUB Peak
1	25	7.5	1	3	7	3	6.925	6.25	15.25	3.4
2	20.5	13	10	15	4	3	8.125	8.625	13.9	3.32
3	26	NA	NA	NA	NA	3	NA	NA	NA	NA

Table 24. C: Distribution of maximal number of antral follicles by size for heifer ID 3892.

Wave #	Size of follicles (mm)					
	3 - 3.9	4 - 4.9	5 - 5.9	6 - 7.9	8 - 9.9	>10
1	23	6	3	3	1	1
2	21	3	2	3	2	2

Figure 25: Alterations in total numbers of antral follicles (≥ 3mm) during the first and ovulatory follicular waves of an estrous cycle and during the early stages of the first follicular wave of the next estrous cycle for heifer ID 3892.

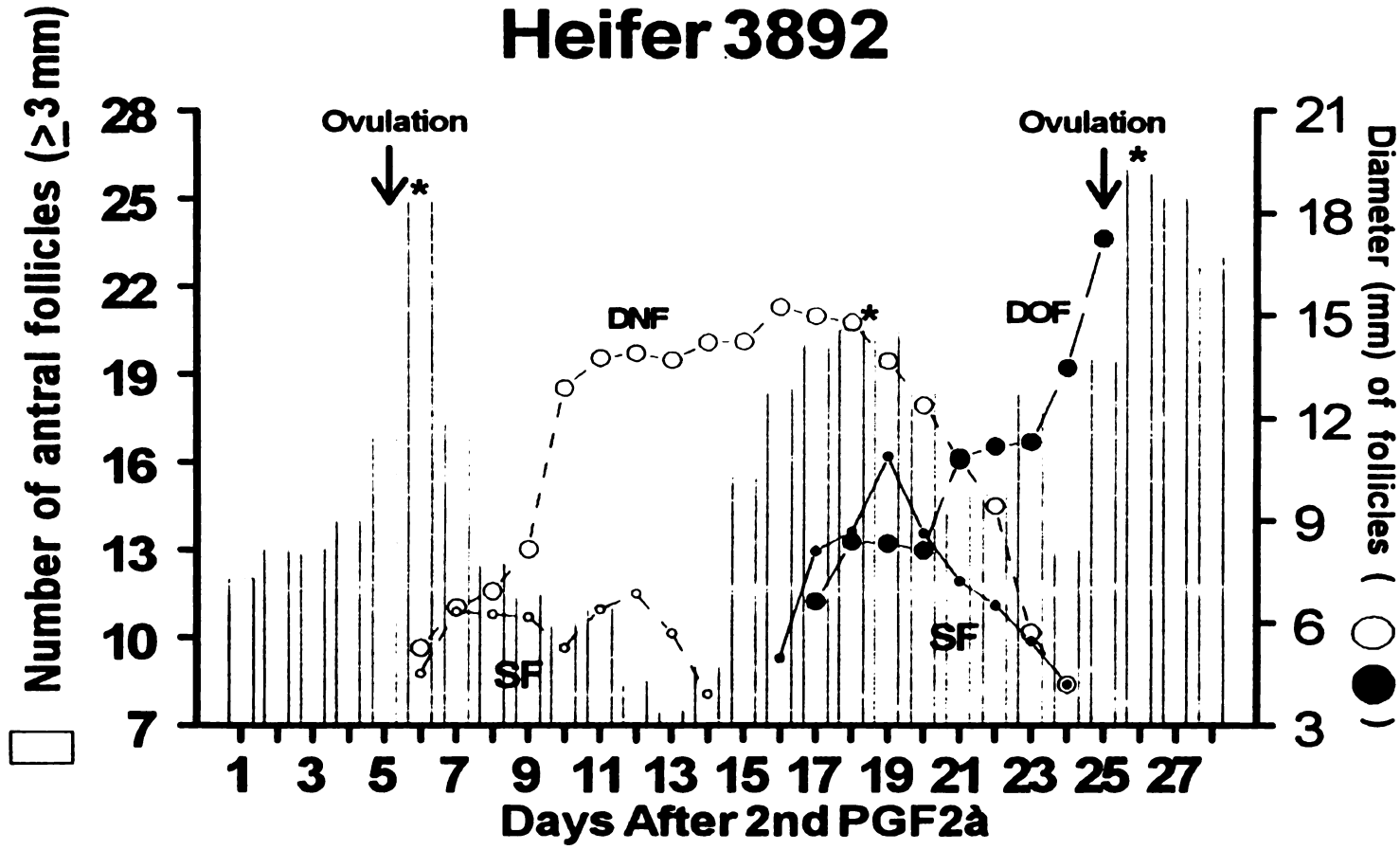


Table 25. A: Animal information for heifer ID 3983.

Animal ID	Age (yr.)	Milking Status	Sire	Dam	Dam's Sire
3893	1	NL	Mar-Thon BW Marshall	Michigan Win Libby 3659	Ladys-Manor Winchester

Table 25. B: Follicle wave dynamics for heifer ID 3983.

Wave #	Maximal Number of Antral Follicles	Baseline Number of Antral Follicles	Day of Emergence (d)	Day of Deviation (d)	Period of Dominance(d)	Classification	Dominant Follicle Growth (mm)			
							DF Dev	SUB Dev	DF Peak	SUB Peak
1	25	10.5	1	1	7	3	6.25	4.8	14.375	3.5
2	25.5	16	8	11	9	3	6.175	6.265	13.8	3.325
3	24	NA	NA	NA	NA	3	NA	NA	NA	NA

Table 25. C: Distribution of maximal number of antral follicles by size for heifer ID 3893.

Wave #	Size of follicles (mm)					
	3 - 3.9	4 - 4.9	5 - 5.9	6 - 7.9	8 - 9.9	>10
1	22	5	4	2	2	1
2	20	5	5	2	2	1

Figure 26: Alterations in total numbers of antral follicles (≥ 3mm) during the first and ovulatory follicular waves of an estrous cycle and during the early stages of the first follicular wave of the next estrous cycle for heifer ID 3893.

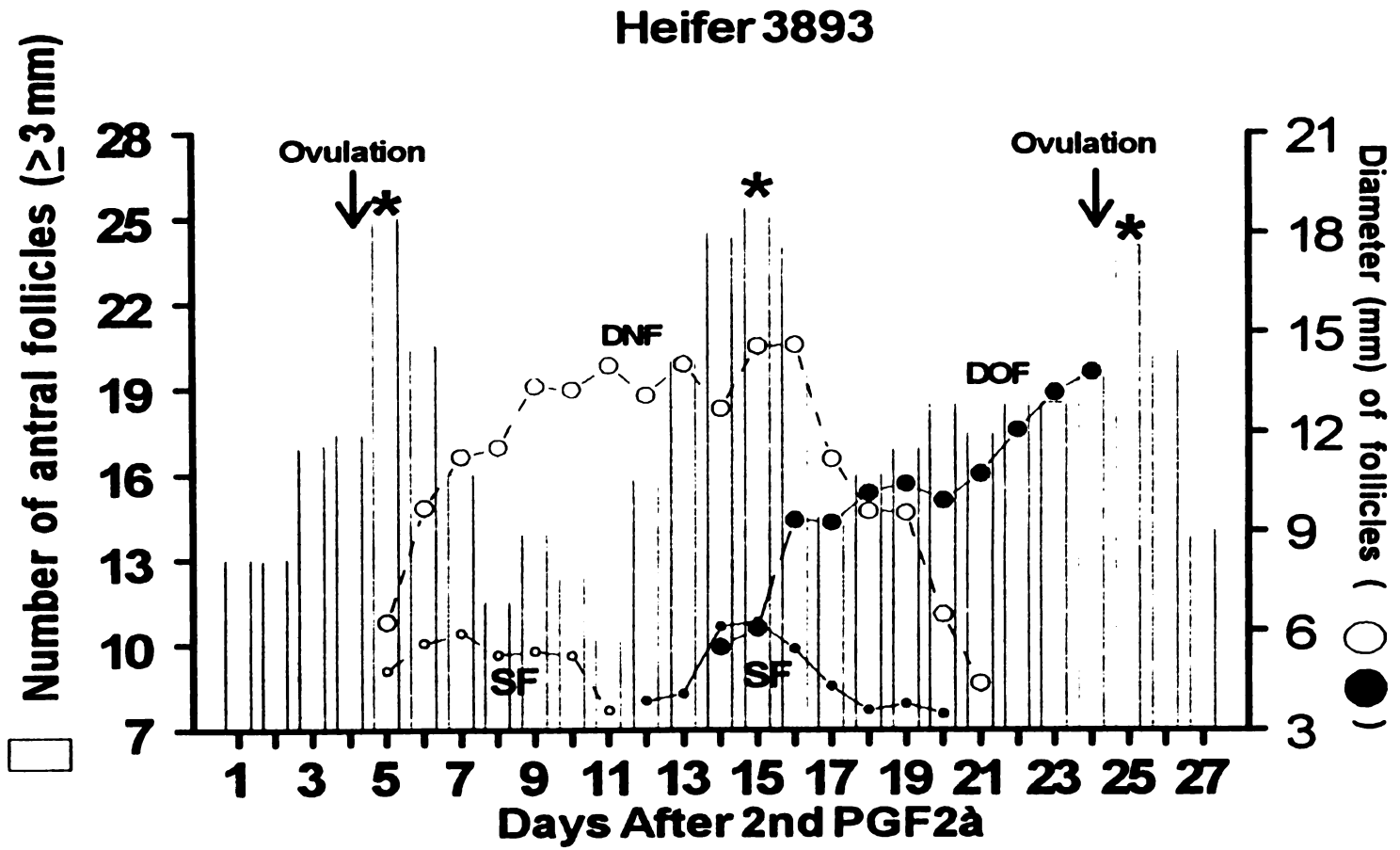


Table 26. A: Animal information for heifer ID 3900.

Animal ID	Age (yr.)	Milking Status	Sire	Dam	Dam's Sire
3900	1	NL	B-Y-U Mandel Bombay	D-P-M MI Pat Briana 3446	Brabant Star Patron

Table 26. B: Follicle wave dynamics for heifer ID 3900.

Wave #	Maximal Number of Antral Follicles	Baseline Number of Antral Follicles	Day of Emergence (d)	Day of Deviation (d)	Period of Dominance(d)	Classification	Dominant Follicle Growth (mm)			
							DF Dev	SUB Dev	DF Peak	SUB Peak
1	23	11	1	3	6	3	7.925	7.35	14.6	3.78
2	20	9	10	10	9	3	3.65	3.3	17.4	3.75
3	21	NA	NA	NA	NA	3	NA	NA	NA	NA

Table 26. C: Distribution of maximal number of antral follicles by size for heifer ID 3900.

Wave #	Size of follicles (mm)					
	3 - 3.9	4 - 4.9	5 - 5.9	6 - 7.9	8 - 9.9	>10
1	20	6	4	3	1	1
2	22	5	4	2	1	2

Figure 27: Alterations in total numbers of antral follicles (≥ 3mm) during the first and ovulatory follicular waves of an estrous cycle and during the early stages of the first follicular wave of the next estrous cycle for heifer ID 3900.

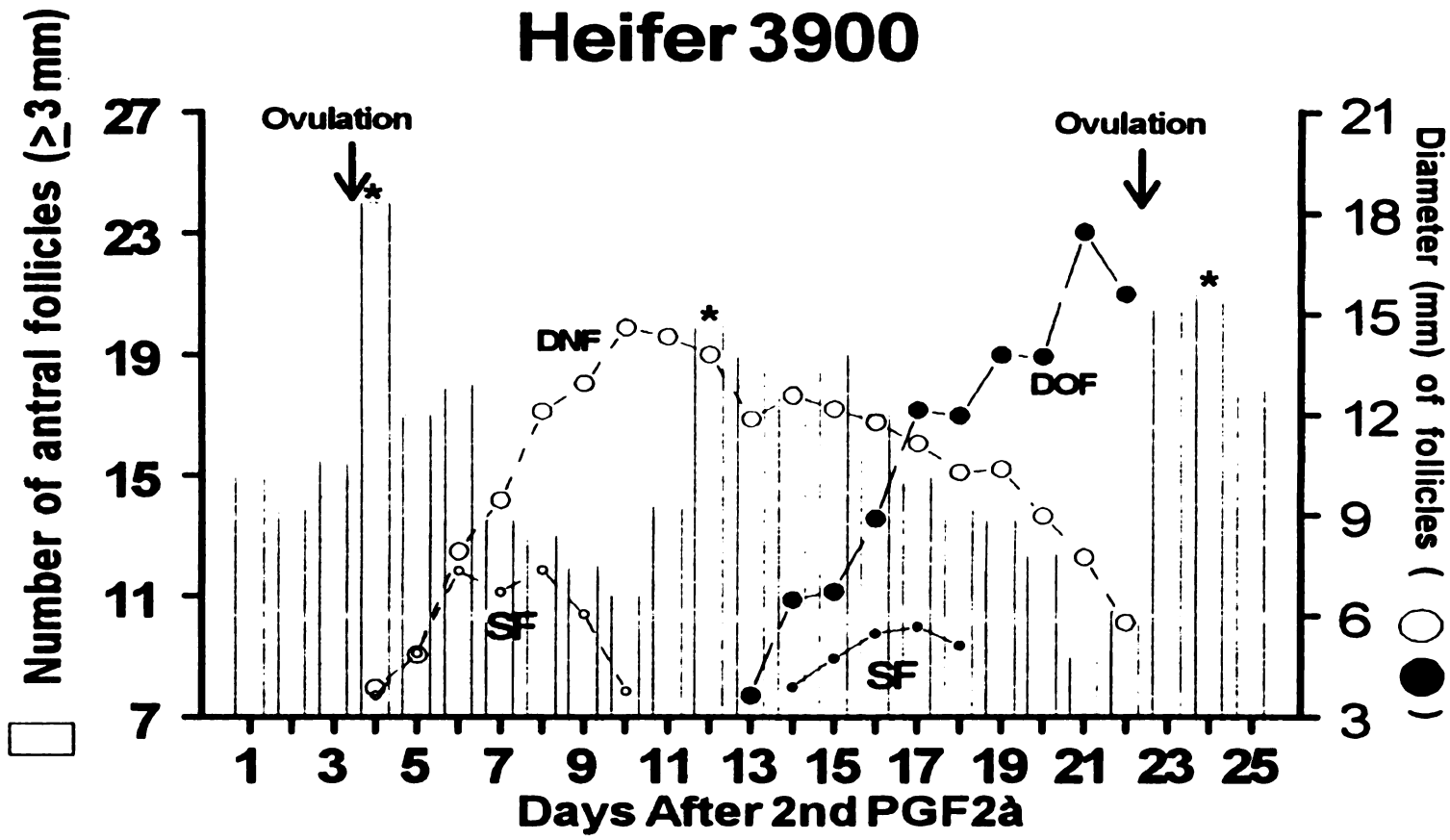


Table 27. A: Animal information for heifer 3905.

Animal ID	Age (yr.)	Milking Status	Sire	Dam	Dam's Sire
3905	1	NL	Michigan Metro Odie 9597	NA	NA

Table 27. B: Follicle wave dynamics for heifer ID 3905.

Wave #	Maximal Number of Antral Follicles	Baseline Number of Antral Follicles	Day of Emergence (d)	Day of Deviation (d)	Period of Dominance(d)	Classification	Dominant Follicle Growth (mm)			
							DF Dev	SUB Dev	DF Peak	SUB Peak
1	24.5	10	1	2	7	3	5.625	5.7	13.75	3.67
2	22	10	10	13	5	3	7.47	5	15.85	3.75
3	22.5	NA	NA	NA	NA	3	NA	NA	NA	NA

Table 27. B: Distribution of maximal number of antral follicles by size for heifer ID 3905.

Wave #	Size of follicles (mm)					
	3 - 3.9	4 - 4.9	5 - 5.9	6 - 7.9	8 - 9.9	>10
1	20	6	5	4	1	1
2	14	5	3	1	1	1

Figure 28: Alterations in total numbers of antral follicles (≥ 3mm) during the first and ovulatory follicular waves of an estrous cycle and during the early stages of the first follicular wave of the next estrous cycle for heifer ID 3905.

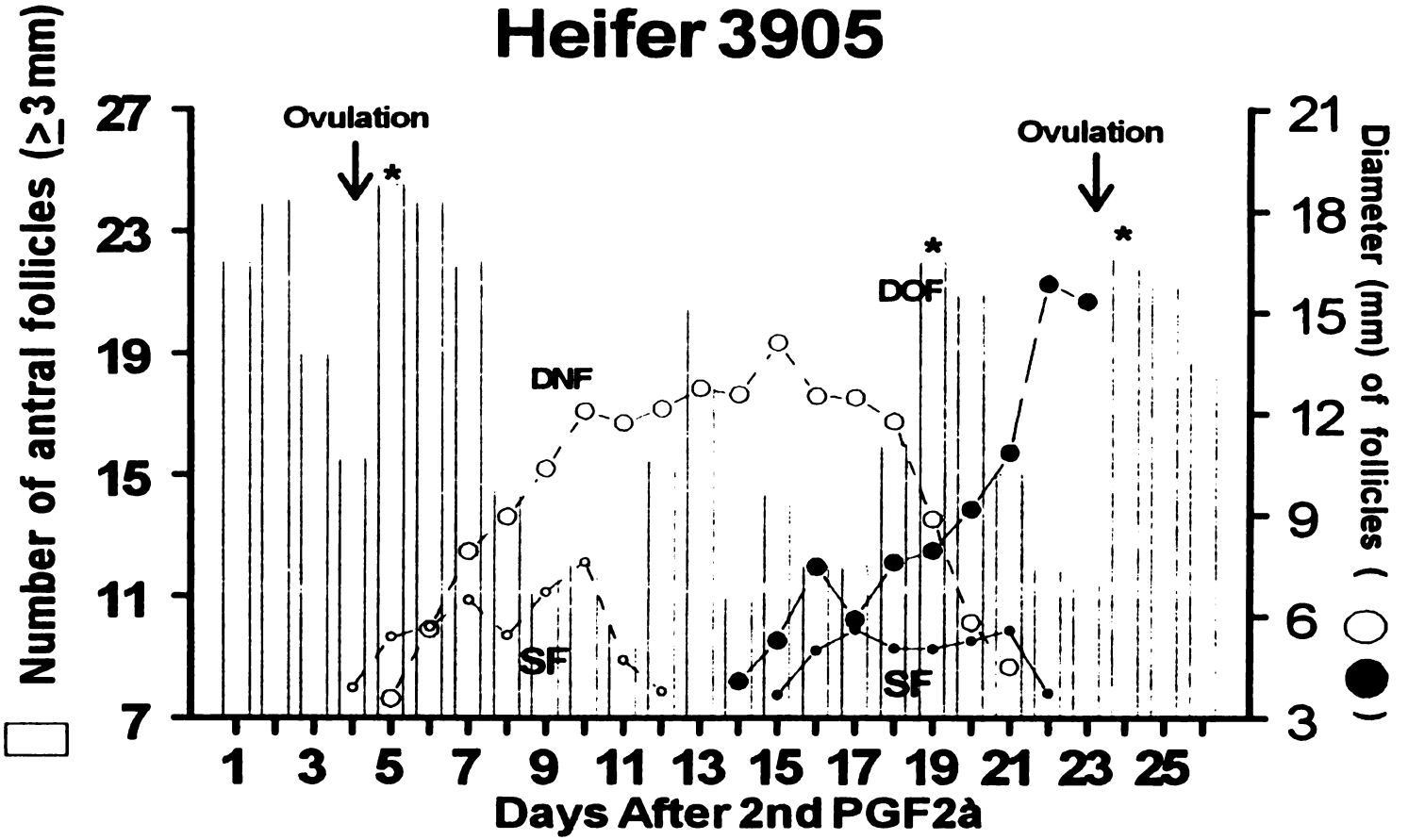


Table 28. A: Animal information for cow ID 3391.

Animal ID	Age (yr.)	Milking Status	Sire	Dam	Dam's Sire
3391	6	L	MJR Blackstar Emory	Michigan Cubby Barb 2990	Osdel-Endevor Bova Cubby

Table 28. B: Follicle wave dynamics for cow ID 3391.

Wave #	Maximal Number of Antral Follicles	Baseline Number of Antral Follicles	Day of Emergence (d)	Day of Deviation (d)	Period of Dominance(d)	Classification	Dominant Follicle Growth (mm)			
							DF Dev	SUB Dev	DF Peak	SUB Peak
1	45	24	1	2	7	4	7.27	6.32	18.875	3.7
2	42	21	11	11	7	4	4.2	4.2	18.4	3.98
3	47.5	NA	NA	NA	NA	4	NA	NA	NA	NA

Table 28. C: Distribution of maximal number of antral follicles by size for cow ID 3391.

Wave #	Size of follicles (mm)					
	3 - 3.9	4 - 4.9	5 - 5.9	6 - 7.9	8 - 9.9	>10
1	32	7	5	3	3	2
2	30	8	5	8	2	2

Figure 29: Alterations in total numbers of antral follicles (≥ 3mm) during the first and ovulatory follicular waves of an estrous cycle and during the early stages of the first follicular wave of the next estrous cycle for cow ID 3391.

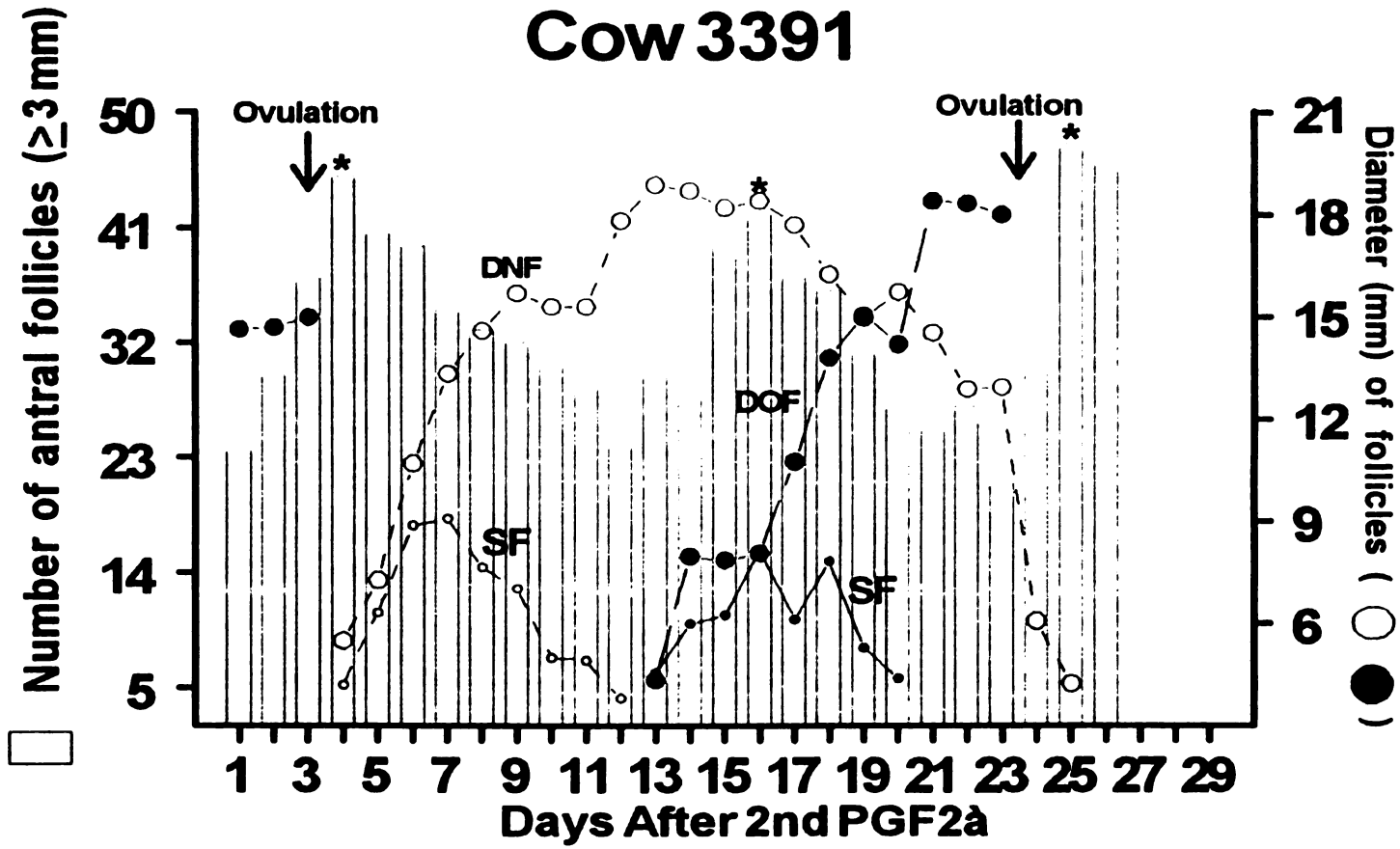


Table 29. A: Animal information for cow ID 3434.

Animal ID	Age (yr.)	Milking Status	Sire	Dam	Dam's Sire
3434	5	NL	Mar-Bil Command Geoffry	Michigan Elton Briana 3197	Emprise Bell Elton

Table 29. B: Follicle wave dynamics for cow ID 3434.

Wave #	Maximal Number of Antral Follicles	Baseline Number of Antral Follicles	Day of Emergence (d)	Day of Deviation (d)	Period of Dominance(d)	Classification	Dominant Follicle Growth (mm)			
							DF Dev	SUB Dev	DF Peak	SUB Peak
1	54	20	1	1	7	4	7.53	6.53	20.23	3.78
2	49	31	10	12	7	4	8.68	7.85	14.95	3.65
3	50	NA	NA	NA	NA	4	NA	NA	NA	NA

Table 29. C: Distribution of maximal number of antral follicles by size for cow ID 3434.

Wave #	Size of follicles (mm)					
	3 - 3.9	4 - 4.9	5 - 5.9	6 - 7.9	8 - 9.9	>10
1	48	8	5	3	2	1
2	44	5	4	2	2	1

Figure 30: Alterations in total numbers of antral follicles (≥ 3mm) during the first and ovulatory follicular waves of an estrous cycle and during the early stages of the first follicular wave of the next estrous cycle for cow ID 3434.

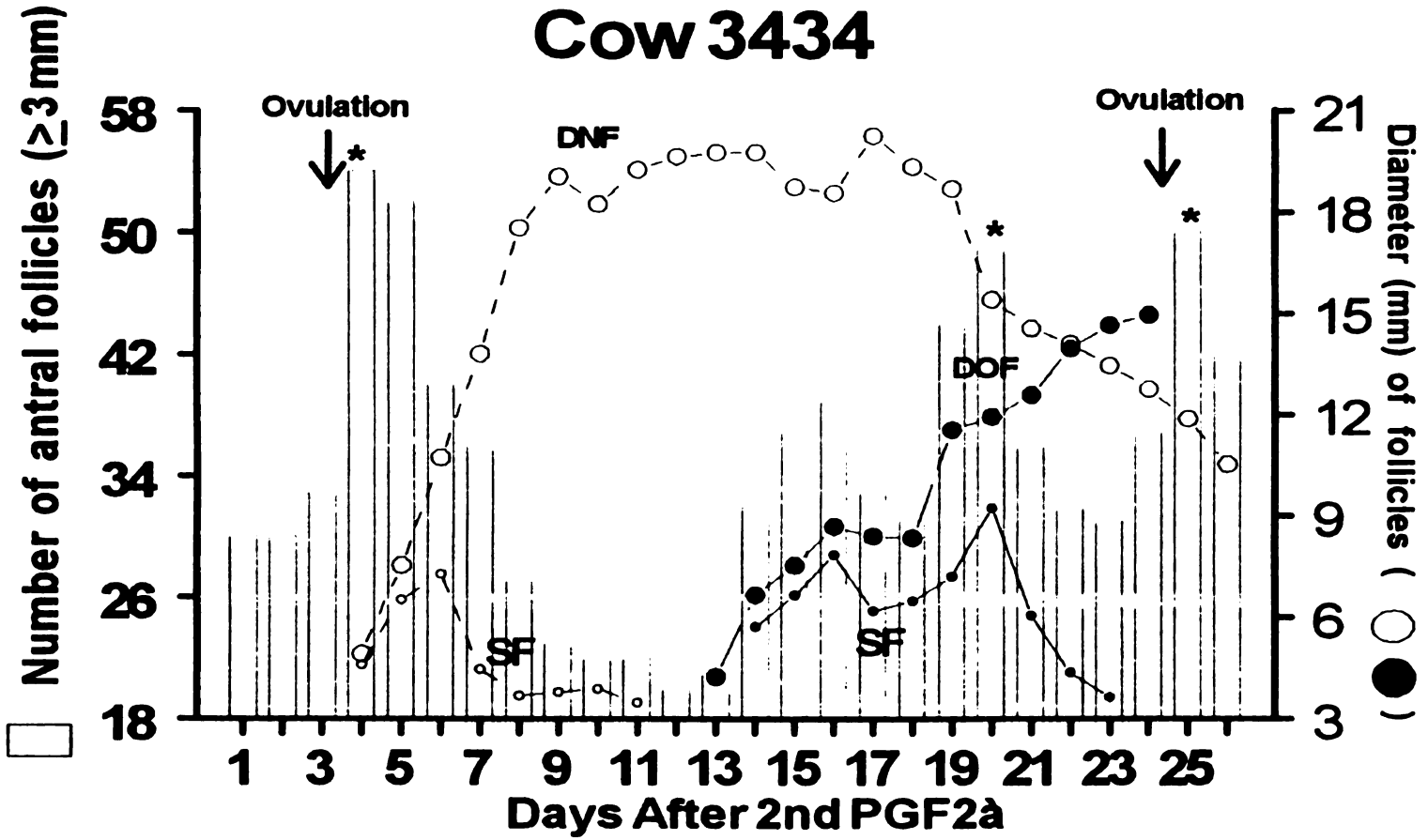


Table 30. A: Animal information for cow ID 3500.

Animal ID	Age (yr.)	Milking Status	Sire	Dam	Dam's Sire
3500	4	NL	H-R-Z Tesk Brandon	Michigan Extreme Maggie 3302	Wormont Extreme

Table 30. B: Follicle wave dynamics for cow ID 3500.

Wave #	Maximal Number of Antral Follicles	Baseline Number of Antral Follicles	Day of Emergence (d)	Day of Deviation (d)	Period of Dominance(d)	Classification	Dominant Follicle Growth (mm)			
							DF Dev	SUB Dev	DF Peak	SUB Peak
1	22	11	1	NA	NA	4	6.68	4.93	14.43	12.53
2	28	17	9	9	10	4	4.95	3.31	13.88	5.68
3	28	16	19	23	4	4	6.55	7.25	15.15	9.48
4	25	NA	NA	NA	NA	4	NA	NA	NA	NA

Table 30. C: Distribution of maximal number of antral follicles by size for cow ID 3500.

Wave #	Size of follicles (mm)					
	3 - 3.9	4 - 4.9	5 - 5.9	6 - 7.9	8 - 9.9	>10
1	15	7	5	3	2	1
2	23	4	4	3	2	1

Figure 31: Alterations in total numbers of antral follicles (≥ 3mm) during the first and ovulatory follicular waves of an estrous cycle and during the early stages of the first follicular wave of the next estrous cycle for cow ID 3500.

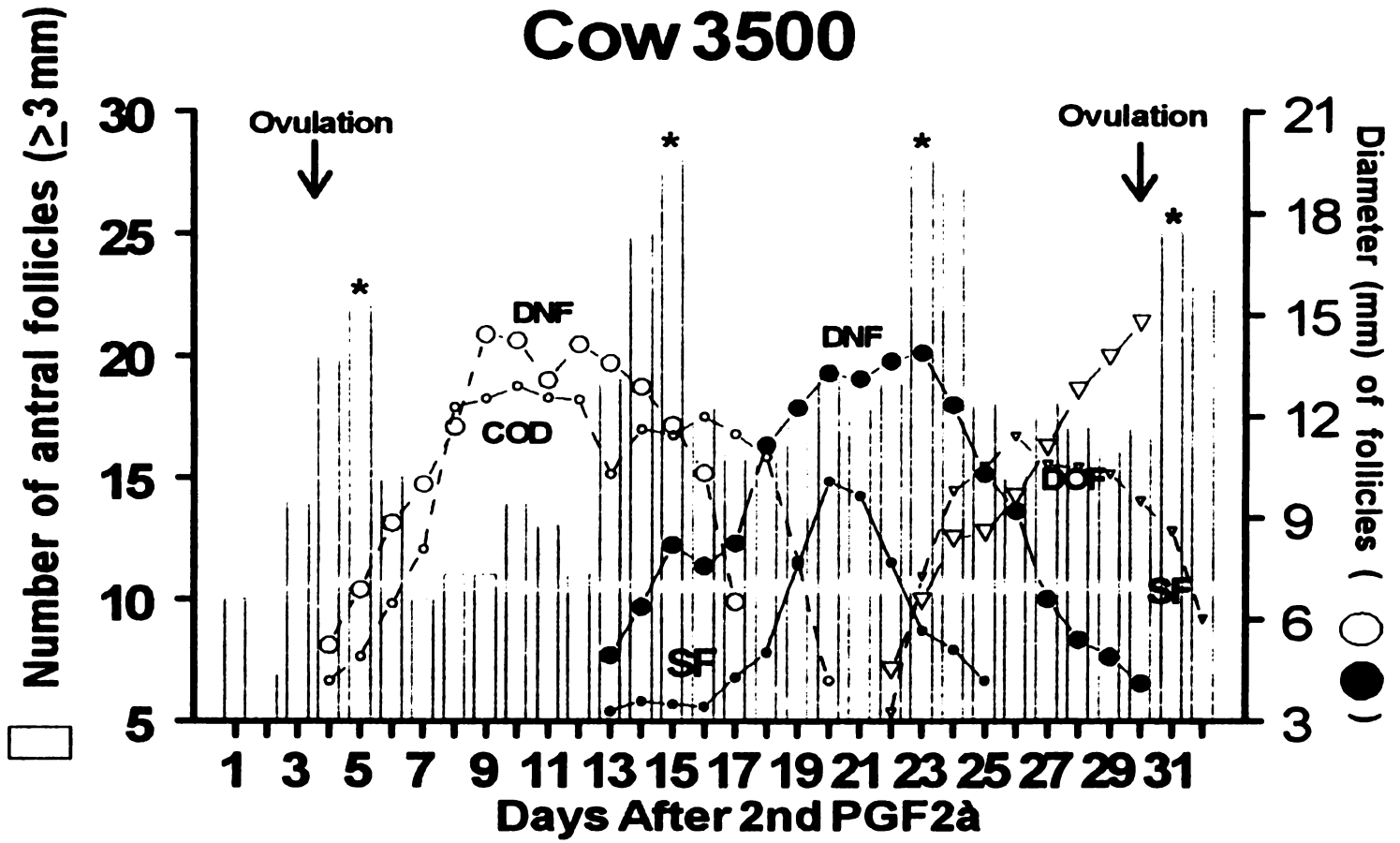


Table 31. A: Animal information for cow ID 3531.

Animal ID	Age (yr.)	Milking Status	Sire	Dam	Dam's Sire
3531	4	L	End-Road Leadman Barlo	Michigan Nic Niclole 3223	United Nick

Table 31. B: Follicle wave dynamics for cow ID 3531.

Wave #	Maximal Number of Antral Follicles	Baseline Number of Antral Follicles	Day of Emergence (d)	Day of Deviation (d)	Period of Dominance(d)	Classification	Dominant Follicle Growth (mm)			
							DF Dev	SUB Dev	DF Peak	SUB Peak
1	28	15.5	1	8	6	4	13.53	13.85	20.43	3.78
2	27.5	12	15	15	9	4	4.7	3.45	10.03	3.34
3	26.5	NA	NA	NA	NA	4	NA	NA	NA	NA

Table 31. C: Distribution of maximal number of antral follicles by size for cow ID 3531.

Wave #	Size of follicles (mm)					
	3 - 3.9	4 - 4.9	5 - 5.9	6 - 7.9	8 - 9.9	>10
1	19	8	4	4	2	3
2	22	5	3	2	1	2

Figure 32: Alterations in total numbers of antral follicles (≥ 3mm) during the first and ovulatory follicular waves of an estrous cycle and during the early stages of the first follicular wave of the next estrous cycle for cow ID 3531.

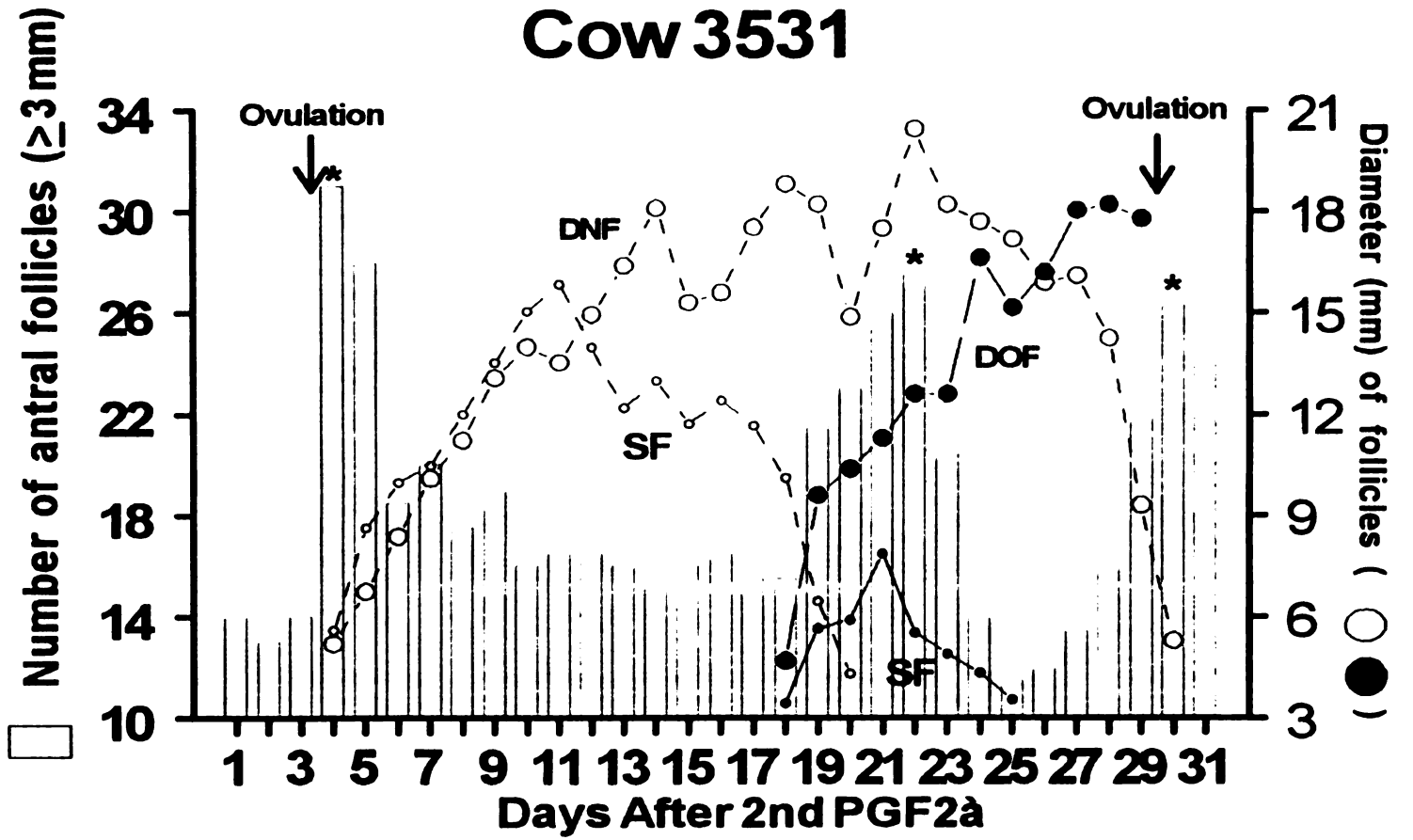


Table 32. A: Animal information for heifer ID 3888.

Animal ID	Age (yr.)	Milking Status	Sire	Dam	Dam's Sire
3888	1	NL	Regancrest Mascot Dello	Michigan Leif Romilda 3571	Block-Bros As Leif

Table 32. B: Follicle wave dynamics for heifer ID 3888.

Wave #	Maximal Number of Antral Follicles	Baseline Number of Antral Follicles	Day of Emergence (d)	Day of Deviation (d)	Period of Dominance(d)	Classification	Dominant Follicle Growth (mm)			
							DF Dev	SUB Dev	DF Peak	SUB Peak
1	40.5	24	1	2	8	4	3.7	3.7	14.03	3.8
2	41.5	25	11	11	8	4	5.28	4.6	14.35	3.3
3	30.5	NA	NA	NA	NA	4	NA	NA	NA	NA

Table 32. C: Distribution of maximal number of antral follicles by size for heifer ID 3888.

Wave #	Size of follicles (mm)					
	3 - 3.9	4 - 4.9	5 - 5.9	6 - 7.9	8 - 9.9	>10
1	42	7	5	4	1	1
2	37	5	3	3	1	2

Figure 33: Alterations in total numbers of antral follicles (≥ 3mm) during the first and ovulatory follicular waves of an estrous cycle and during the early stages of the first follicular wave of the next estrous cycle for heifer ID 3888.

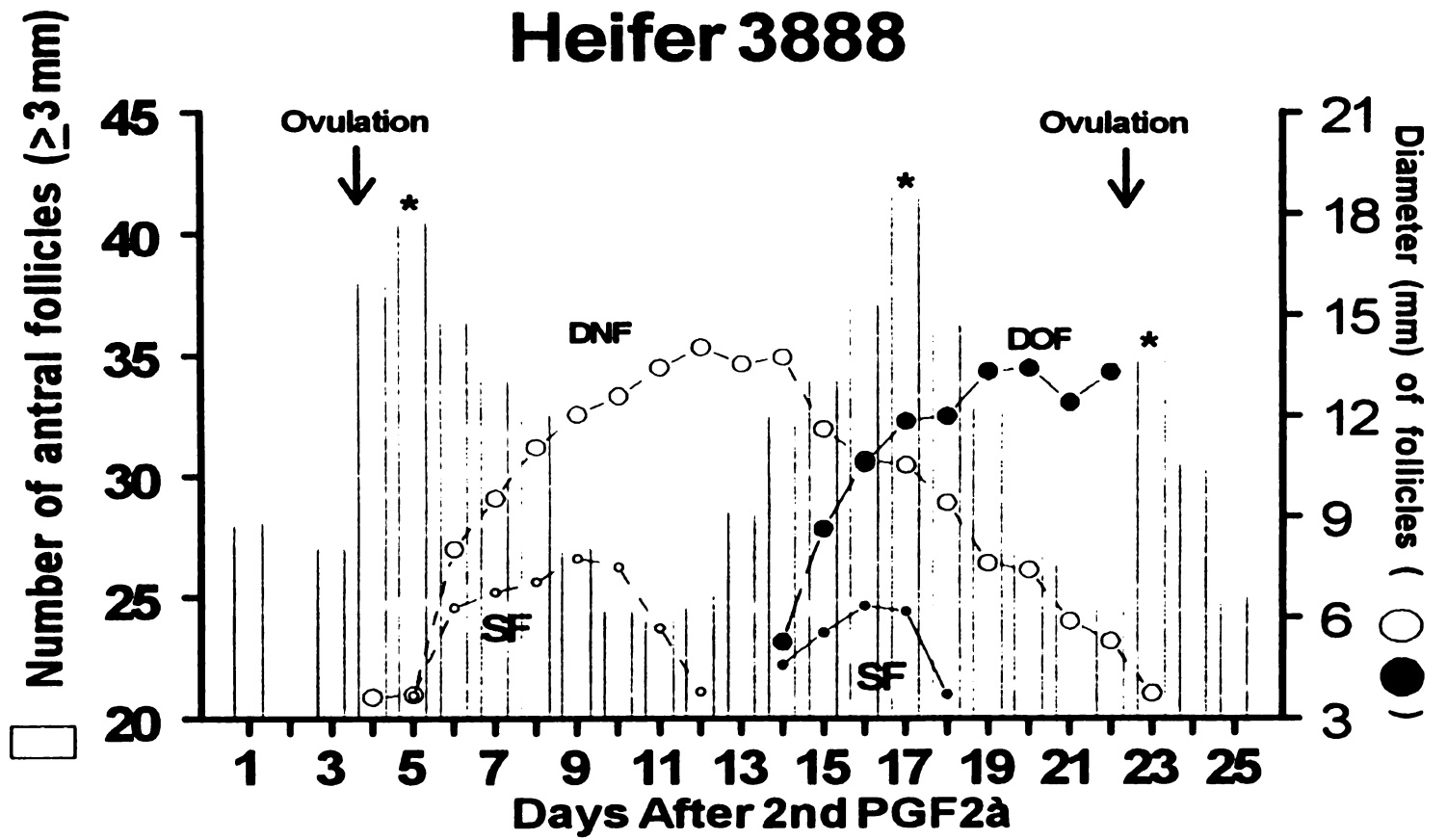


Table 33. A: Animal information for heifer ID 3897.

Animal ID	Age (yr.)	Milking Status	Sire	Dam	Dam's Sire
3897	1	NL	AR-Joy Slocum Adam	Michigan Oro Crunch 3665	Mar-River Bellwood Oreo

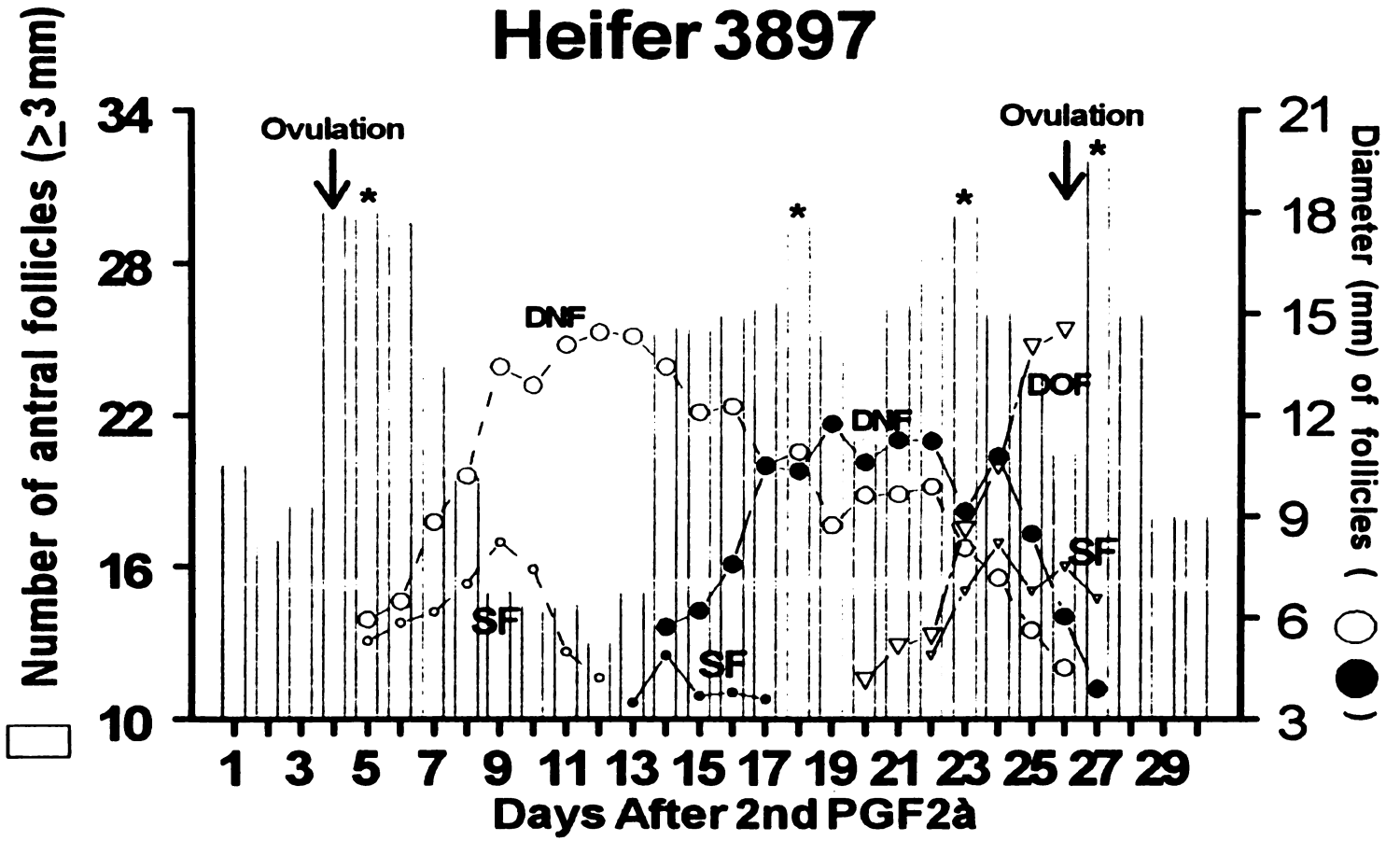
Table 33. B: Follicle wave dynamics for heifer ID 3897.

Wave #	Maximal Number of Antral Follicles	Baseline Number of Antral Follicles	Day of Emergence (d)	Day of Deviation (d)	Period of Dominance(d)	Classification	Dominant Follicle Growth (mm)			
							DF Dev	SUB Dev	DF Peak	SUB Peak
1	33	13	1	3	7	4	6.48	5.85	14.45	4.23
3	30	20.5	17	19	4	4	5.15	3.68	14.5	7.5
3	29.5	21	11	11	6	4	5.73	4.9	11.23	3.33
4	32	NA	NA	NA	NA	4	NA	NA	NA	NA

Table 33. B: Distribution of maximal number of antral follicles by size for heifer ID 3897.

Wave #	Size of follicles (mm)					
	3 - 3.9	4 - 4.9	5 - 5.9	6 - 7.9	8 - 9.9	>10
1	25	8	4	3	1	1
2	27	5	3	3	1	2
3	26	4	3	3	2	2

Figure 34: Alterations in total numbers of antral follicles (≥ 3mm) during the first and ovulatory follicular waves of an estrous cycle and during the early stages of the first follicular wave of the next estrous cycle for heifer ID 3897.



Part B

Figures for individual animals in Study 2B

Definitions and description for Figures from Study 2B

Alterations in total numbers of antral follicles ($\geq$ 3mm) during the first and ovulatory follicular waves of an estrous cycle and during the early stages of the first follicular wave of the next estrous cycle by a second ultrasound technician of each animal in this study. Each bar represents the average for the total number of antral follicles in both ovaries determined at each of two daily ultrasound sessions (0800, 1800). * Above bar = maximal number of follicles in each wave. Arrows indicate ovulation. Lines represent changes in diameter of the dominant and the largest subordinate follicle for the first and ovulatory wave. DNF = Dominant non-ovulatory follicle, DOF = Dominant ovulatory follicle, SF = Largest subordinate follicle.

Figure 35: Alterations in total numbers of antral follicles (≥ 3mm) during the first and ovulatory follicular waves of an estrous cycle and during the early stages of the first follicular wave of the next estrous cycle for cow ID 3609.

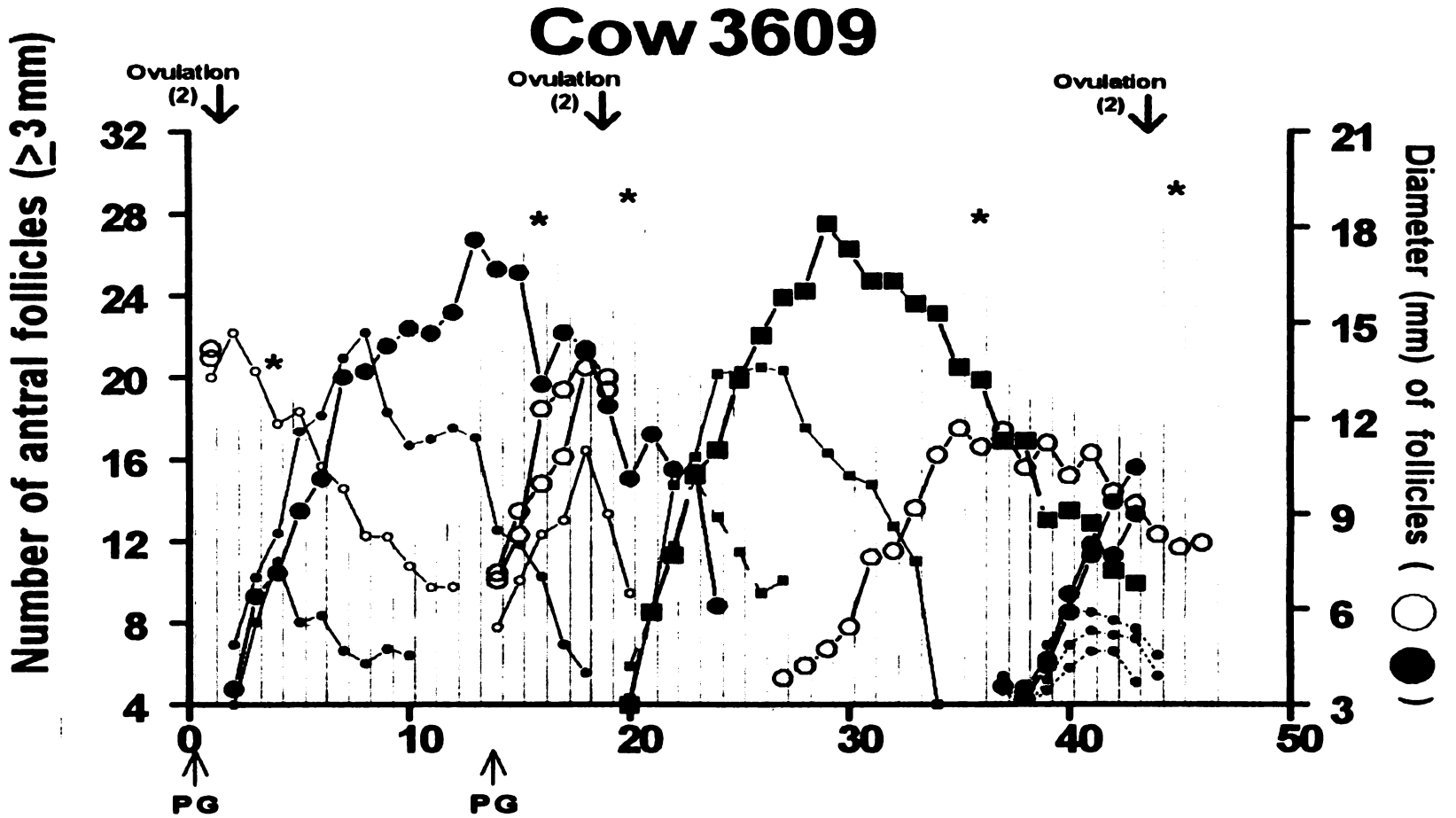


Figure 36: Alterations in total numbers of antral follicles (≥ 3mm) during the first and ovulatory follicular waves of an estrous cycle and during the early stages of the first follicular wave of the next estrous cycle for cow ID 3762.

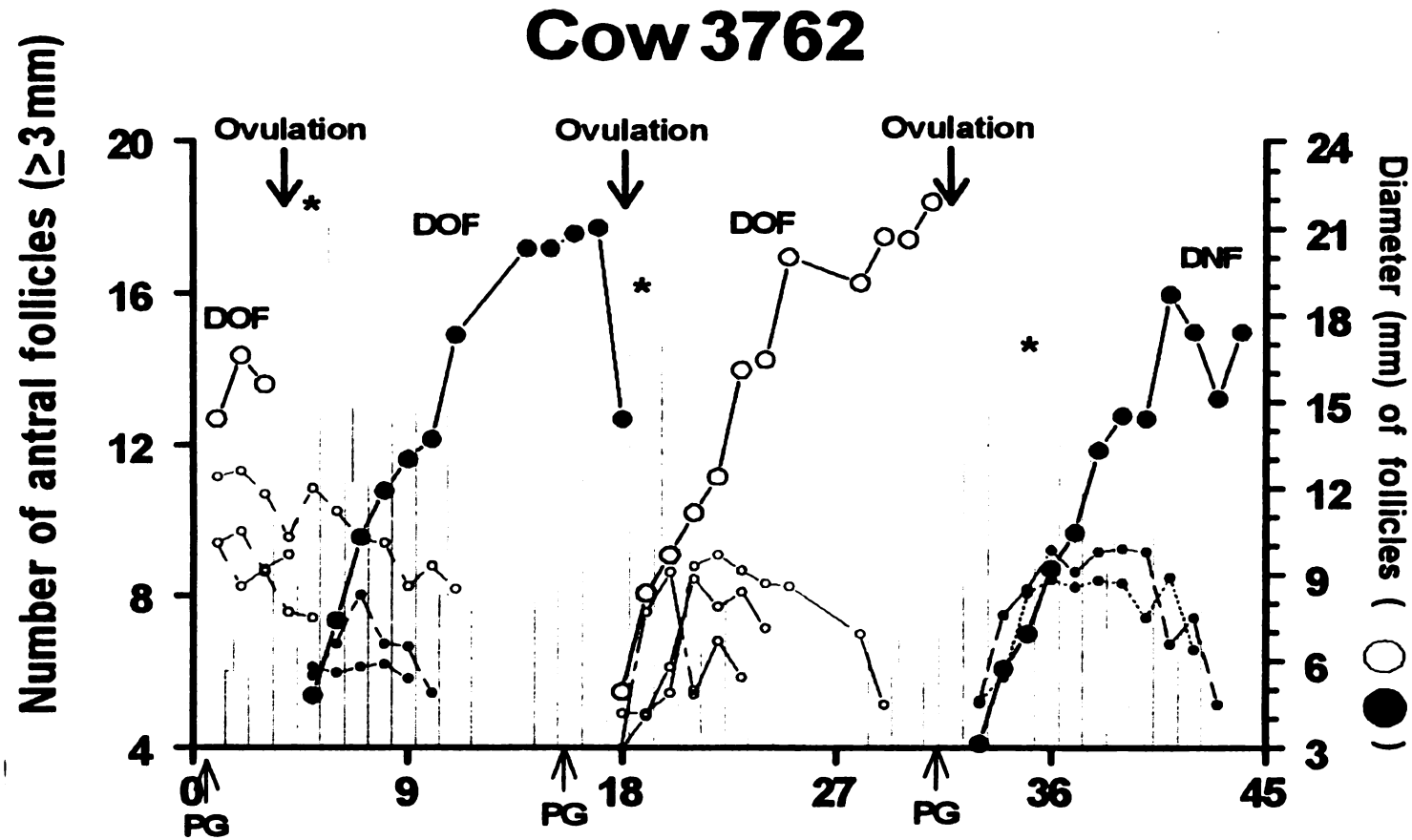


Figure 37: Alterations in total numbers of antral follicles (≥ 3mm) during the first and ovulatory follicular waves of an estrous cycle and during the early stages of the first follicular wave of the next estrous cycle for heifer ID 3976.

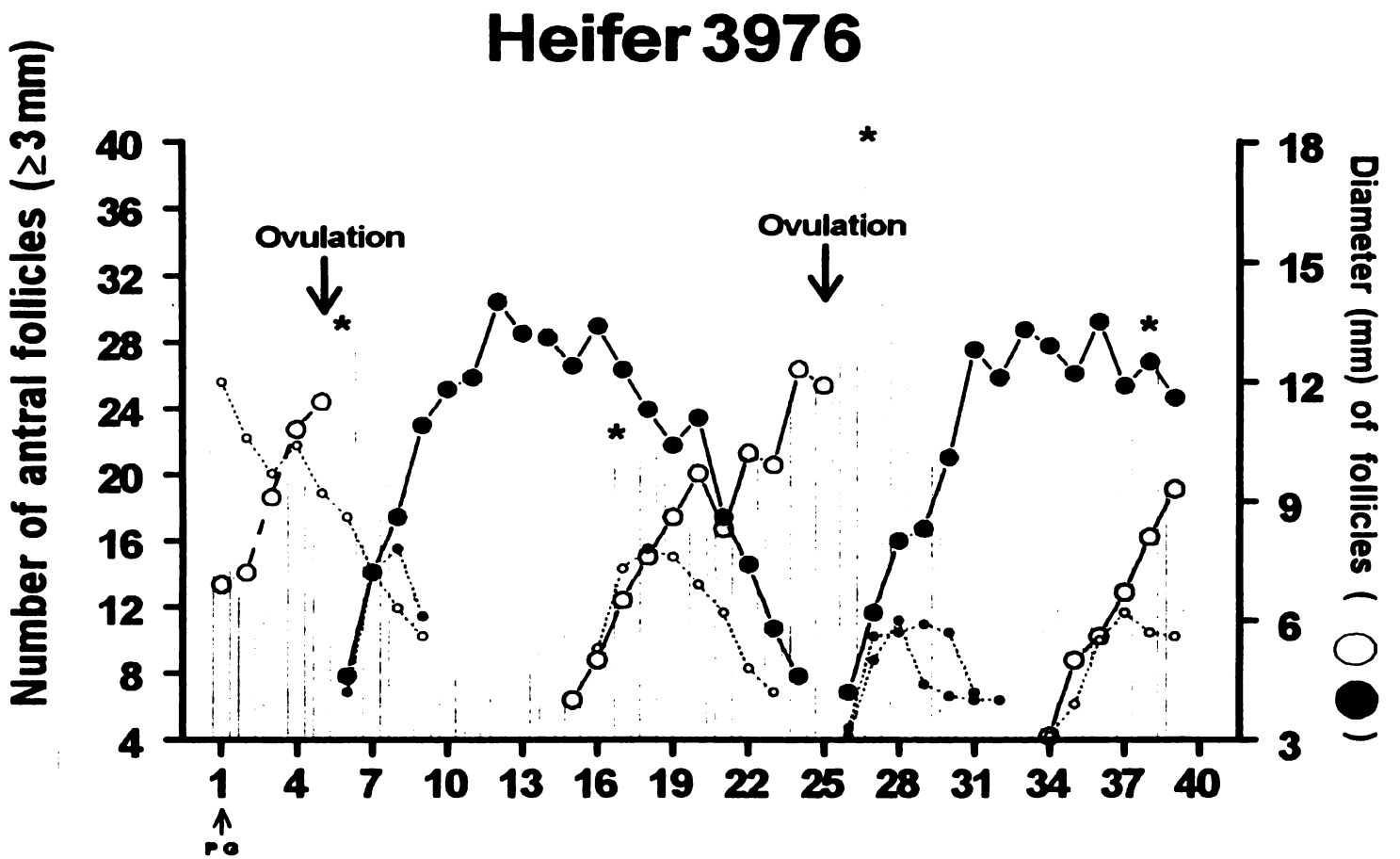


Figure 38: Alterations in total numbers of antral follicles (≥ 3mm) during the first and ovulatory follicular waves of an estrous cycle and during the early stages of the first follicular wave of the next estrous cycle for heifer ID 3977.

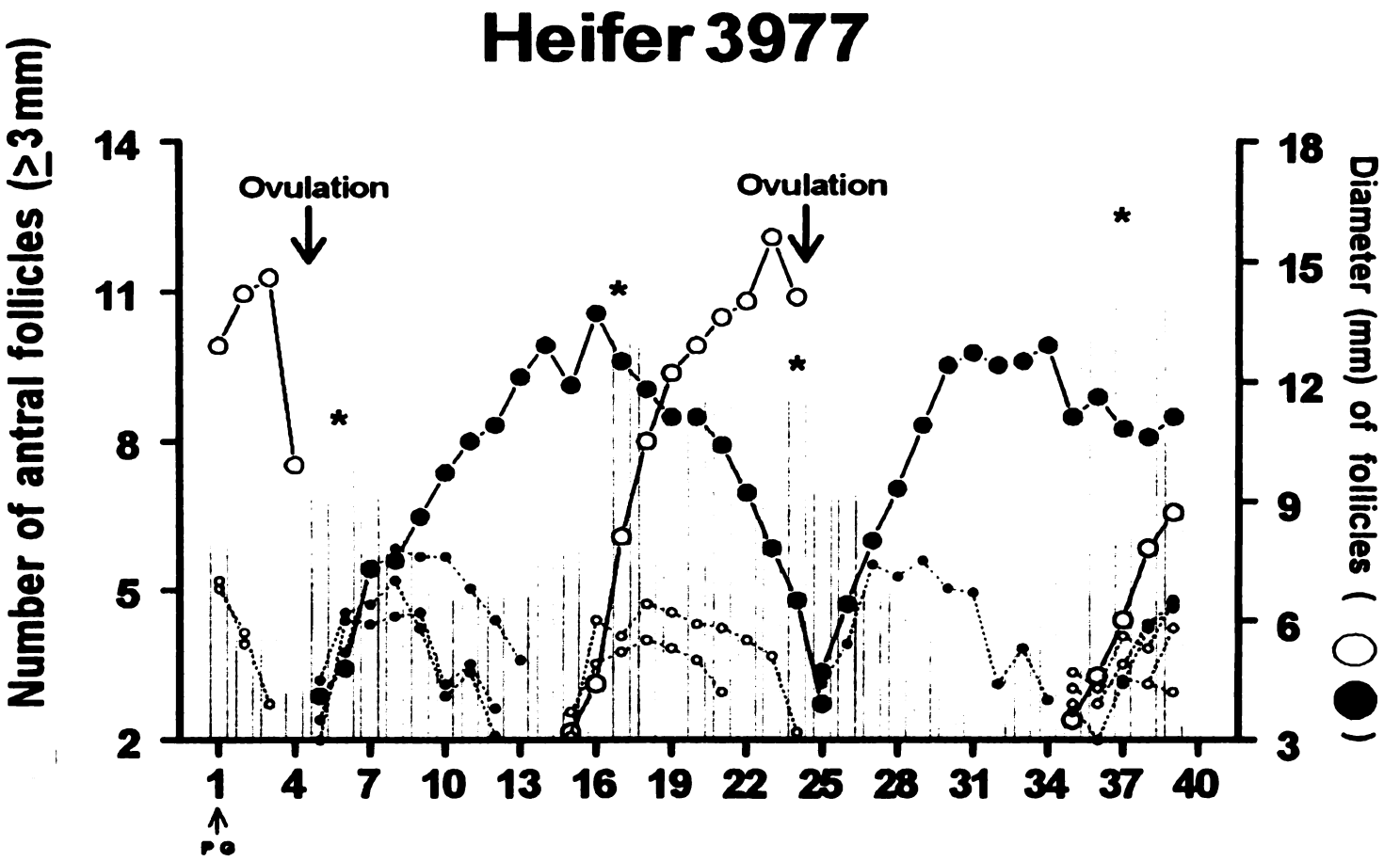


Figure 39: Alterations in total numbers of antral follicles (≥ 3mm) during the first and ovulatory follicular waves of an estrous cycle and during the early stages of the first follicular wave of the next estrous cycle for heifer ID 3980.

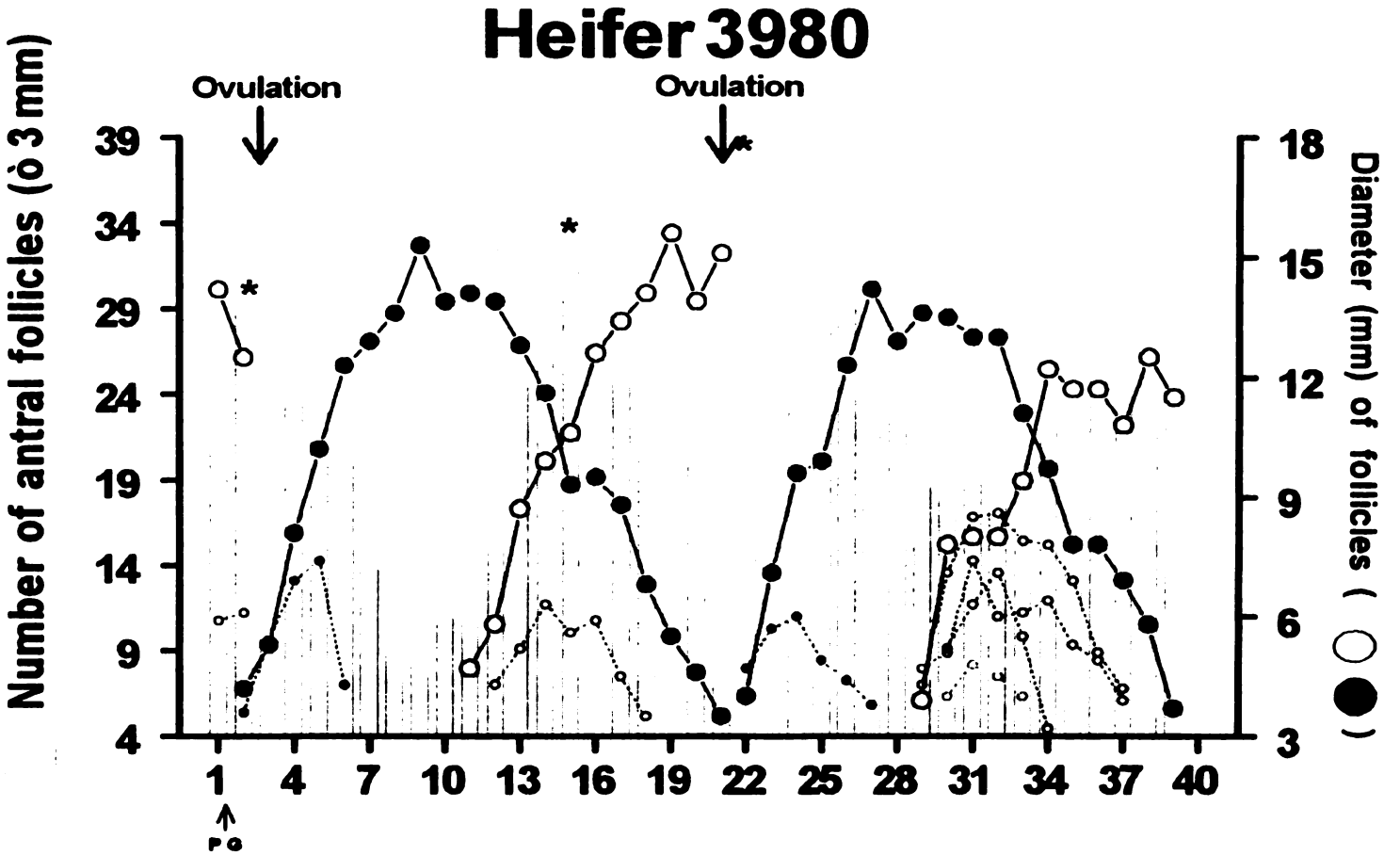
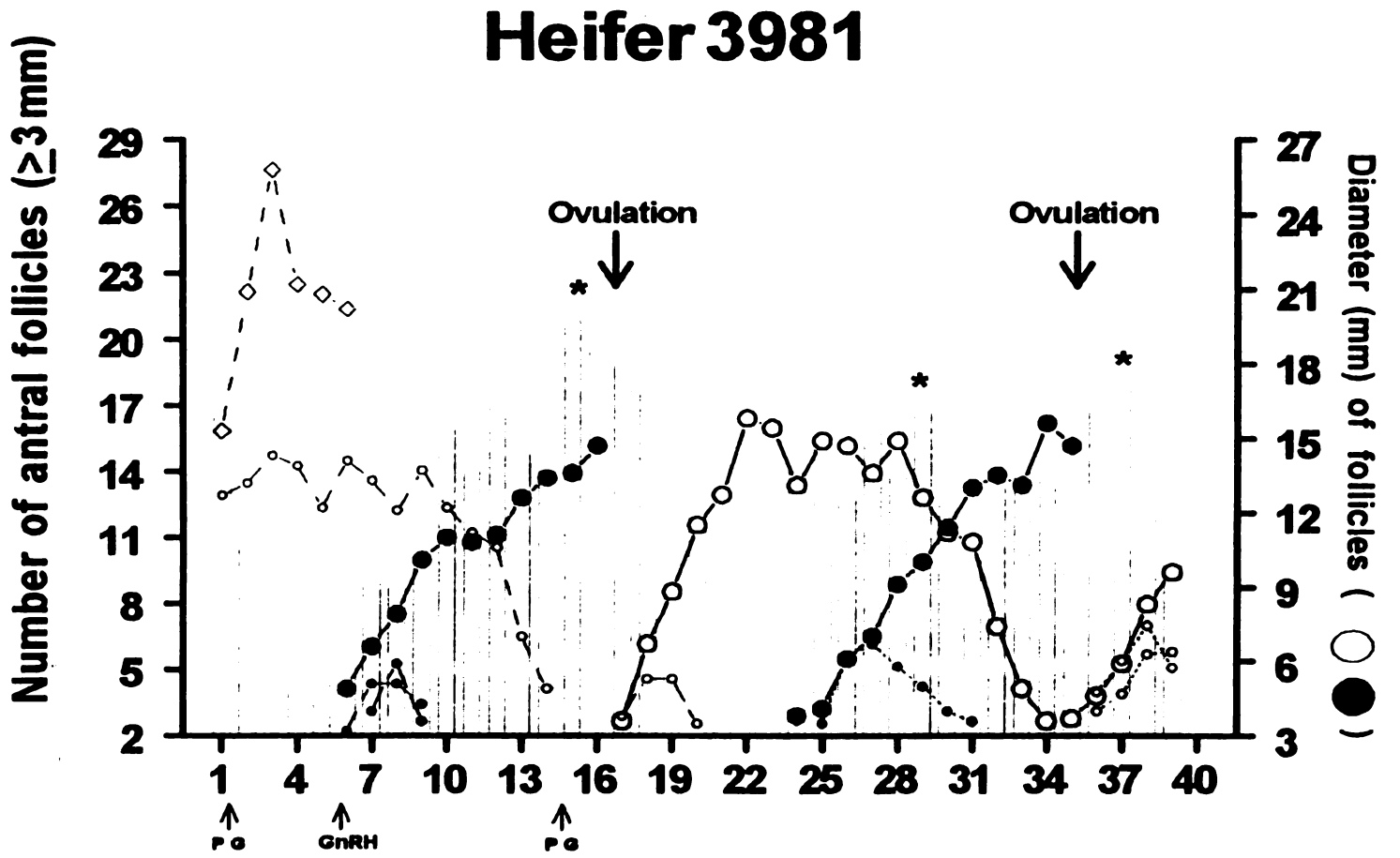


Figure 40: Alterations in total numbers of antral follicles (≥ 3mm) during the first and ovulatory follicular waves of an estrous cycle and during the early stages of the first follicular wave of the next estrous cycle for heifer ID 3981.



Part III

Figures and tables

from study 4

Definitions and description for Figures from study 2B

1. Alterations in total numbers of antral follicles (≥ 3mm) during the first wave of an estrous cycle and bleeding period of each cow in this study. Each bar represents the average for the total number of antral follicles in both ovaries determined at each of two daily ultrasound sessions (0800, 1800). * Above bar = maximal number of follicles in each wave. Arrows indicate ovulation. Lines represent changes in diameter of the dominant and the largest subordinate follicle for the first and ovulatory wave. DNF = Dominant non-ovulatory follicle, DOF = Dominant ovulatory follicle, SF = Largest subordinate follicle.

2. Inhibin-A, estradiol, and FSH profiles aligned to the first FSH surge for each cow in this study. Lines represent changes in concentrations of inhibin-A, estradiol, and FSH. Arrows indicate ovulation.

Table 35. Ages and maximal numbers of antral follicles after ovulation for three consecutive synchronizations.

		Maximal number of antral follicles			
Cow ID	Age	Synch 1	Synch 2	Bleed	AVG[1]
3238	5y 2mo	34	37	44	38.3 ± 2.9
3456	4y 10mo	17	13	16	15.3 ± 1.2
3495	4y 4mo	36	32	31	33.0 ± 1.5
3539	4y 3mo	15[2]	18	14	15.6 ± 1.2
3531	4y 6mo	33	29	32	31.3 ± 1.2
3612	4y 7mo	13	13	11	12.3 ± 0.6
3783	3y 9mo	14	16	23[3]	17.6 ± 2.7
3674	4y 9mo	35	38	37	36.6 ± 0.9

[1] Average ± standard error of the mean

[2] Did not synchronize

[3] Became cystic GnRH administered

Figure 41. Alterations in total numbers of antral follicles (≥ 3mm) during the first wave of an estrous cycle of cow ID 3238.

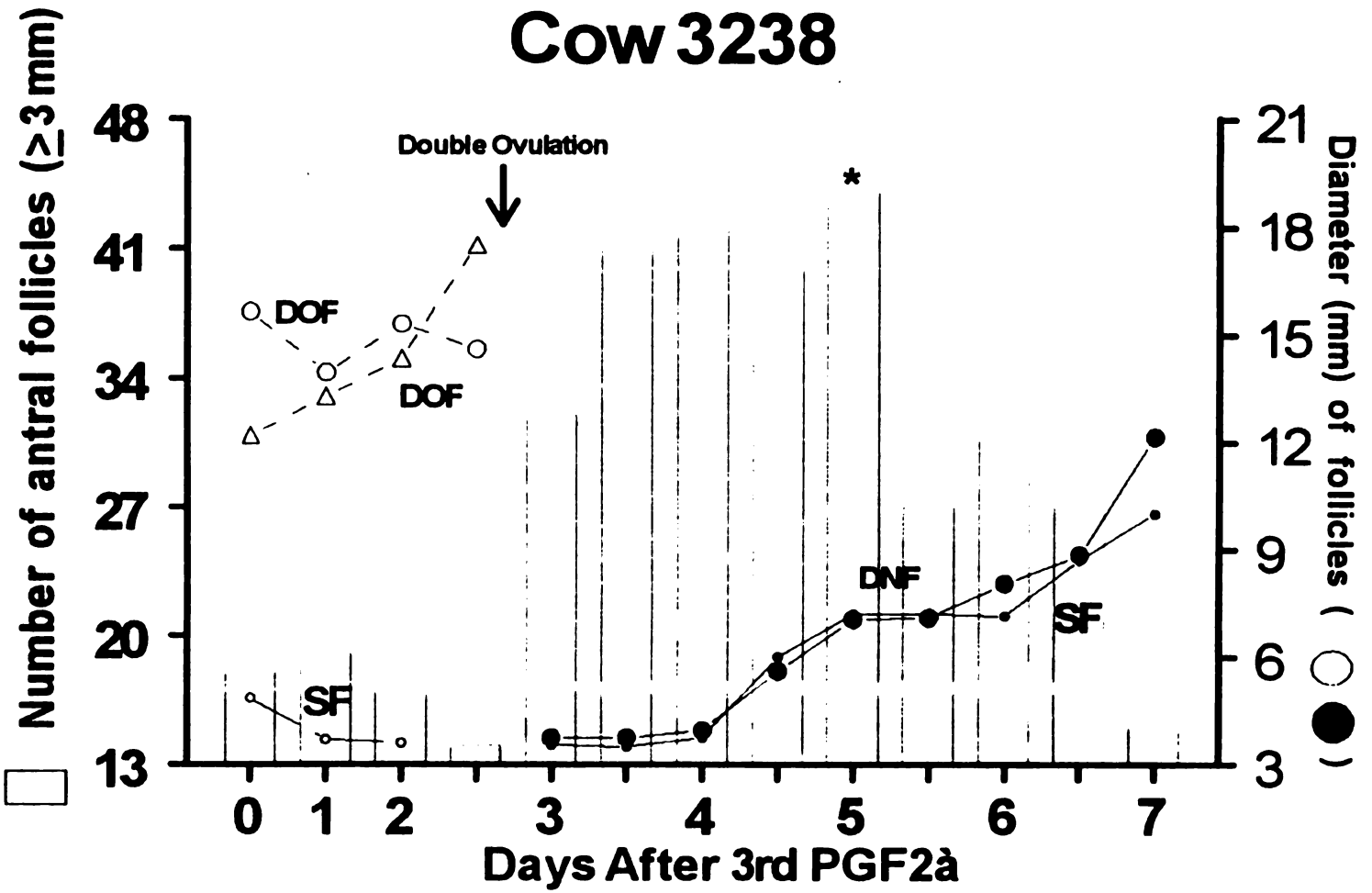


Figure 42. Inhibin-A, estradiol, and FSH profiles aligned to the first FSH surge for cow ID 3238.

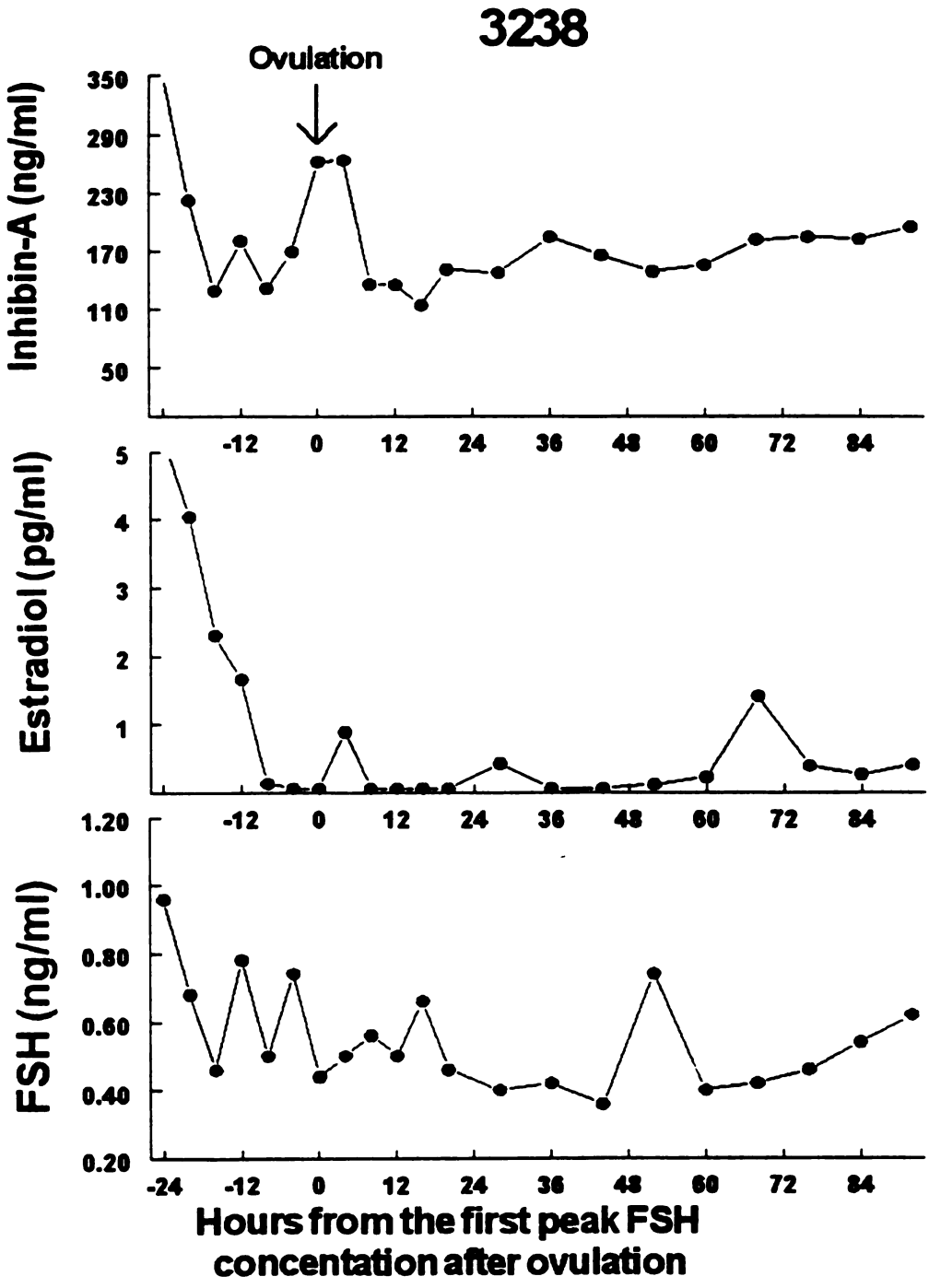


Figure 43. Alterations in total numbers of antral follicles (≥ 3mm) during the first wave of an estrous cycle of cow ID 3495.

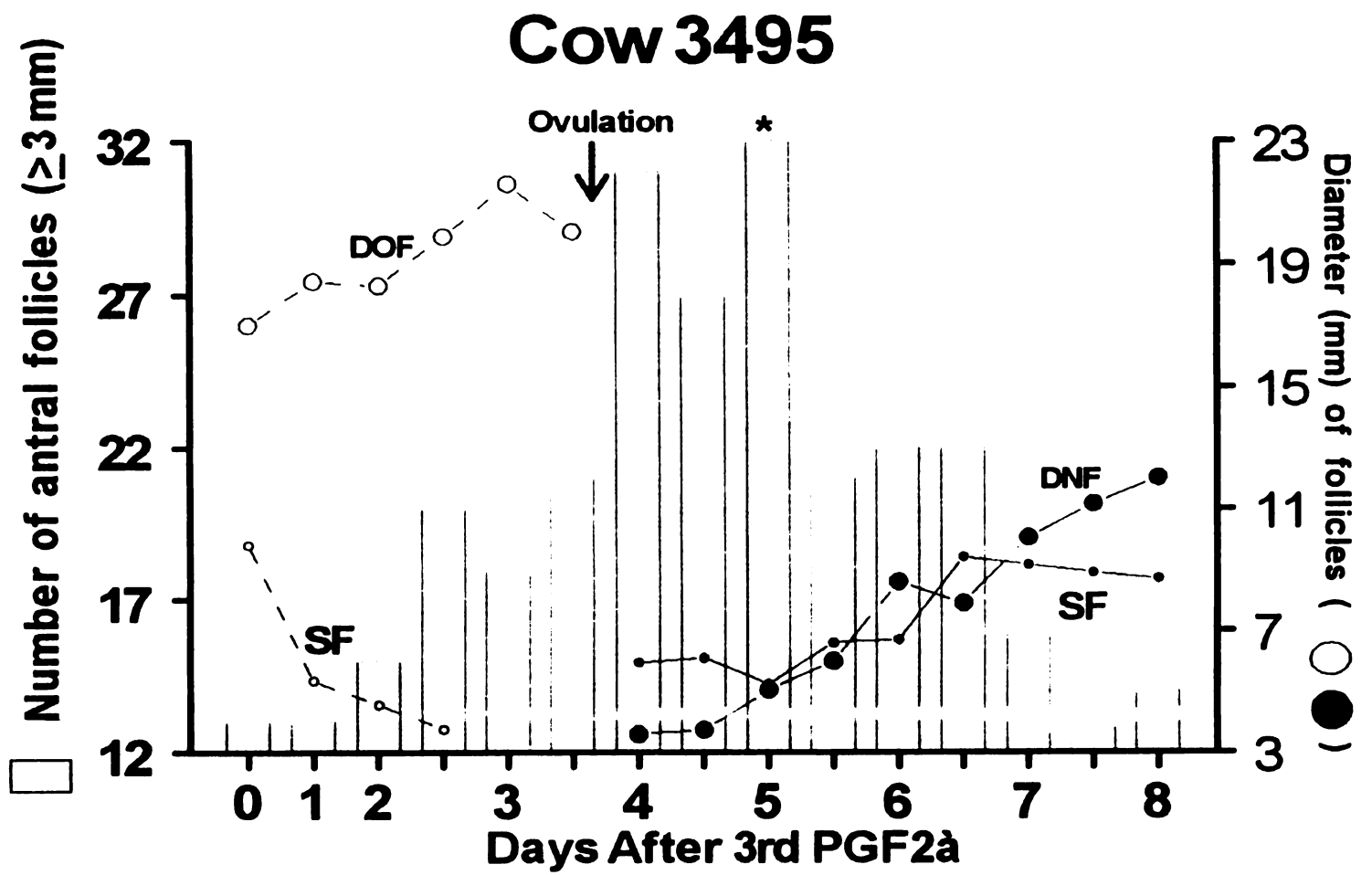


Figure 44. Inhibin-A, estradiol, and FSH profiles aligned to the first FSH surge for cow ID 3495.

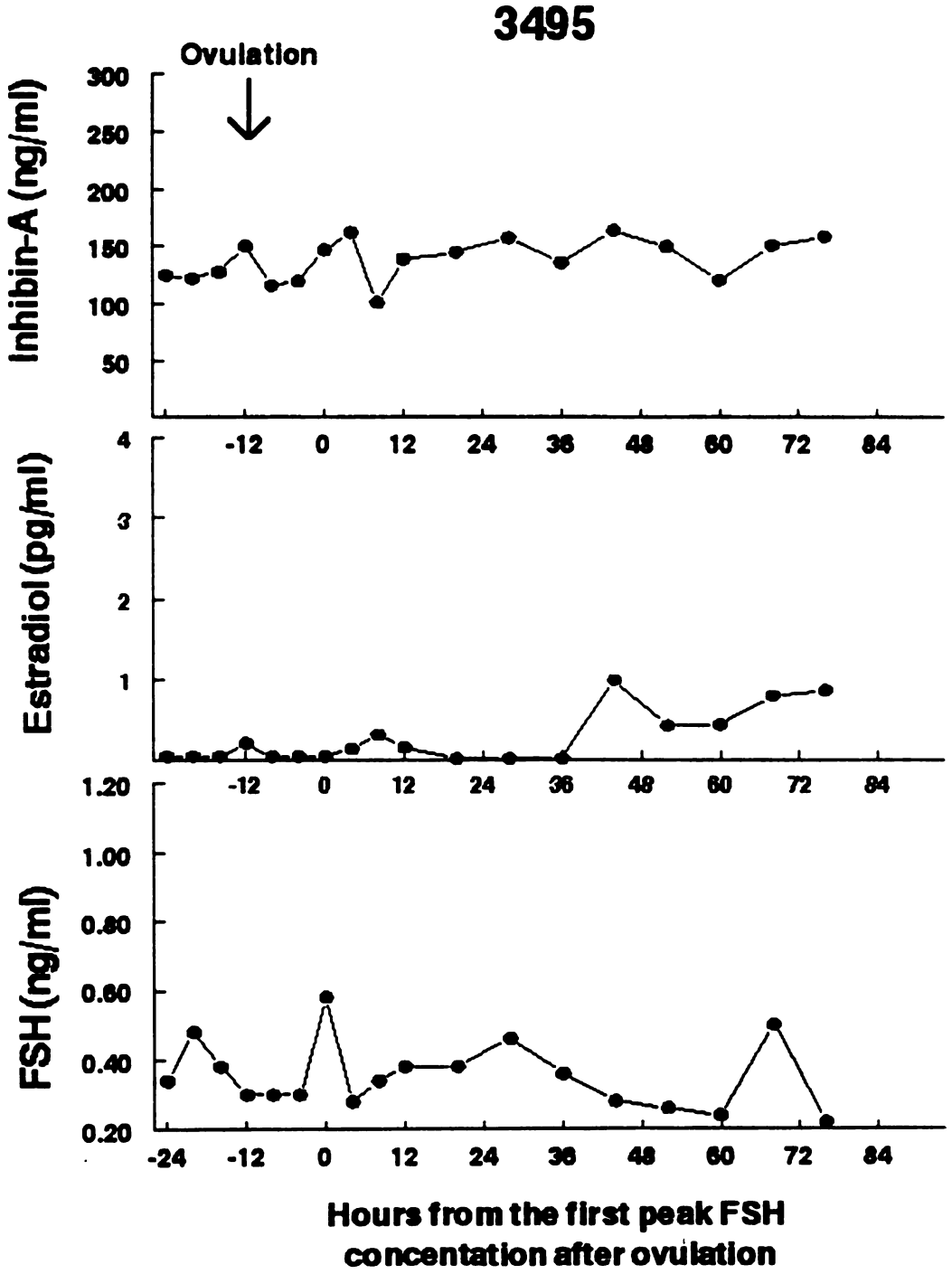


Figure 45. Alterations in total numbers of antral follicles (≥ 3mm) during the first wave of an estrous cycle of cow ID 3531.

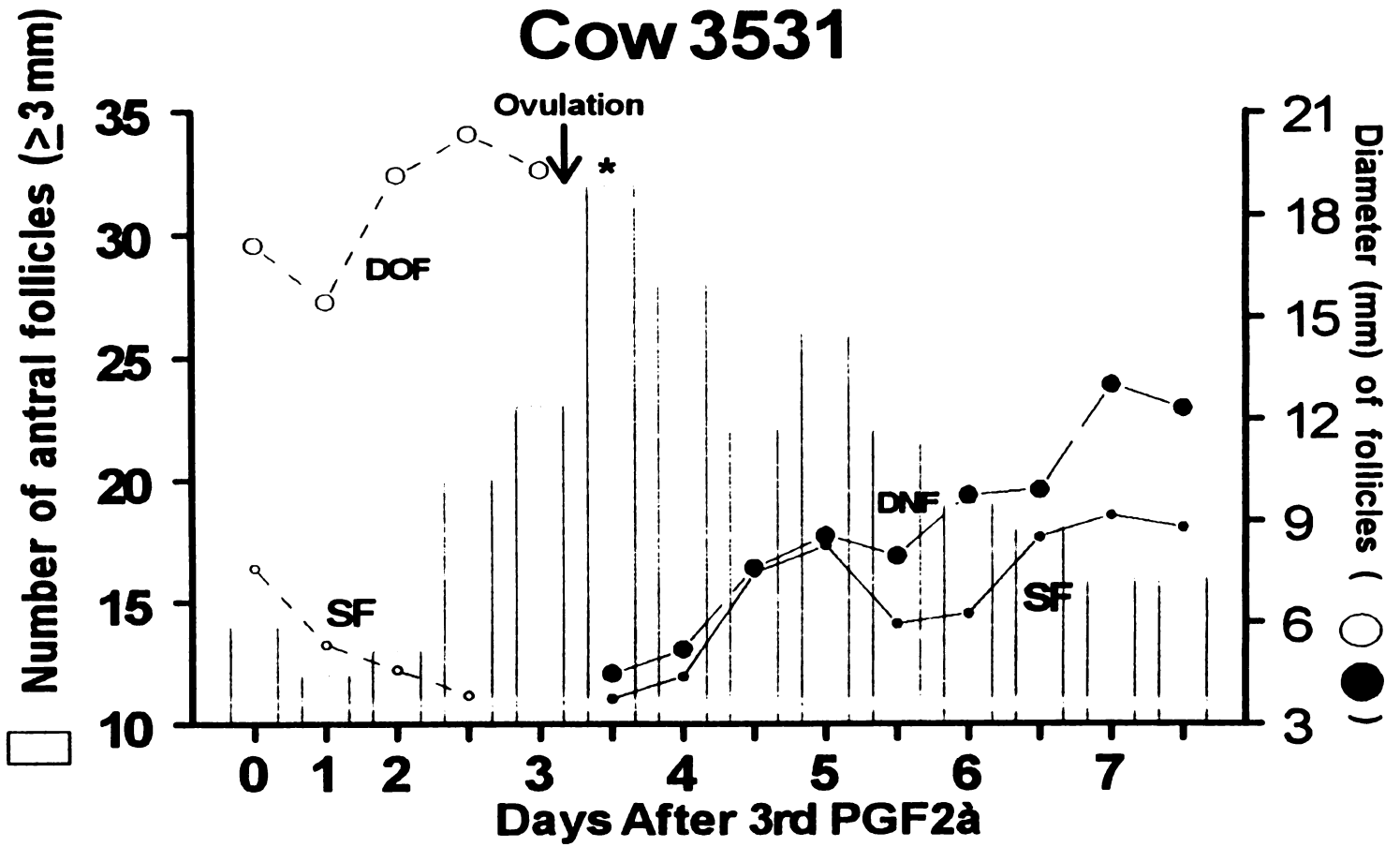


Figure 46. Inhibin-A, estradiol, and FSH profiles aligned to the first FSH surge for cow ID 3531.

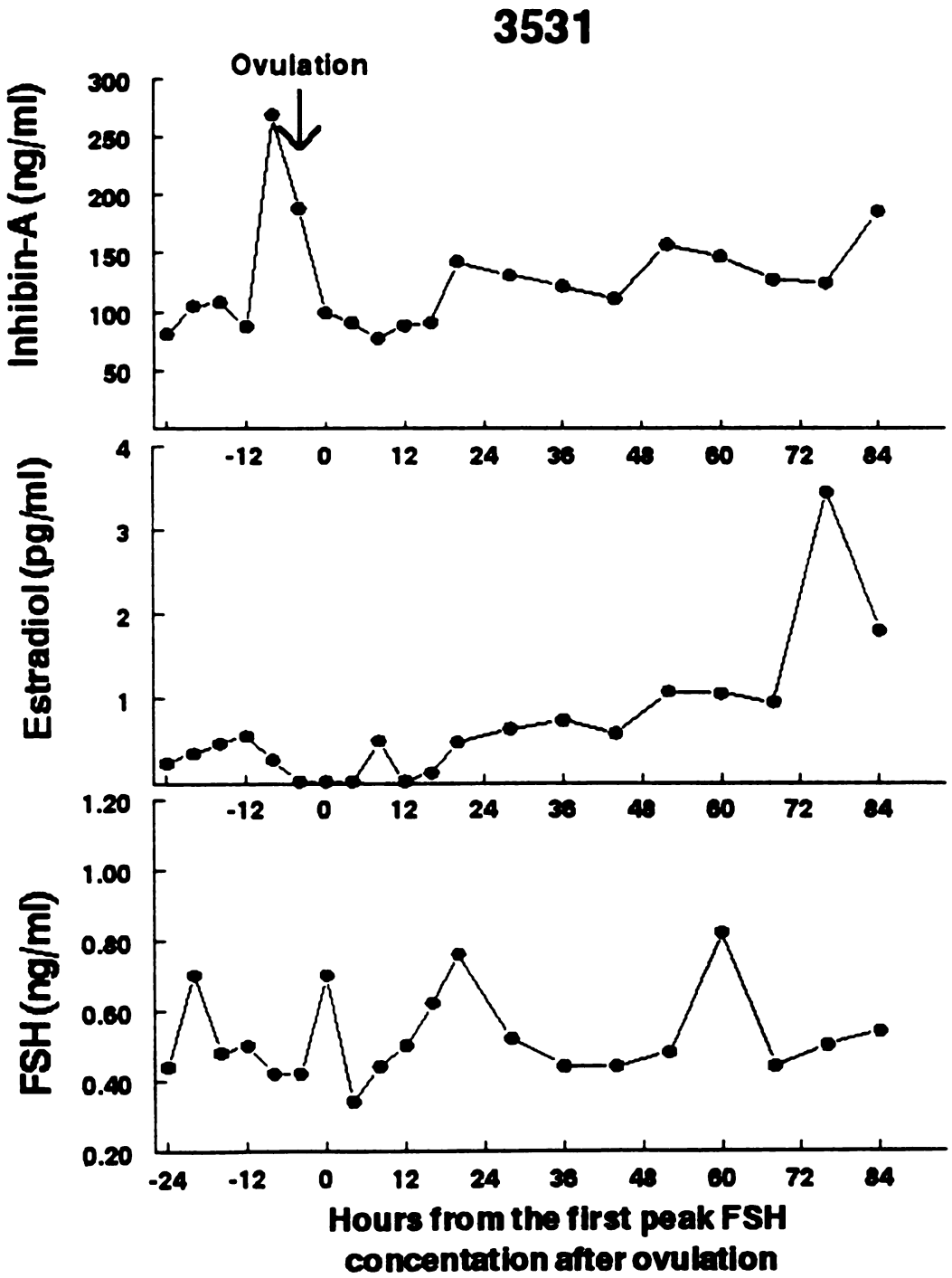


Figure 47. Alterations in total numbers of antral follicles (≥ 3mm) during the first wave of an estrous cycle of cow ID 3674.

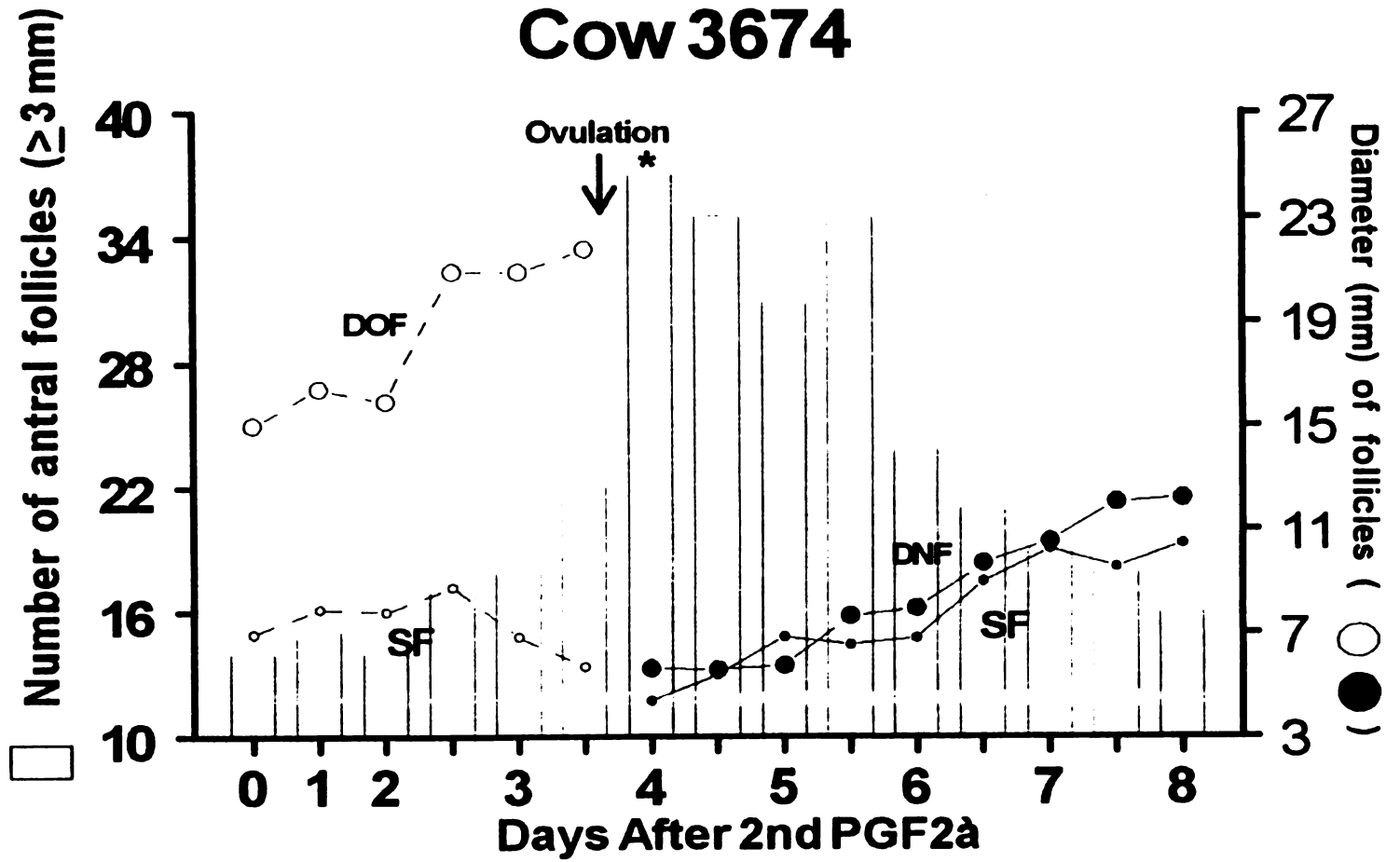


Figure 48. Inhibin-A, estradiol, and FSH profiles aligned to the first FSH surge for cow ID 3674.

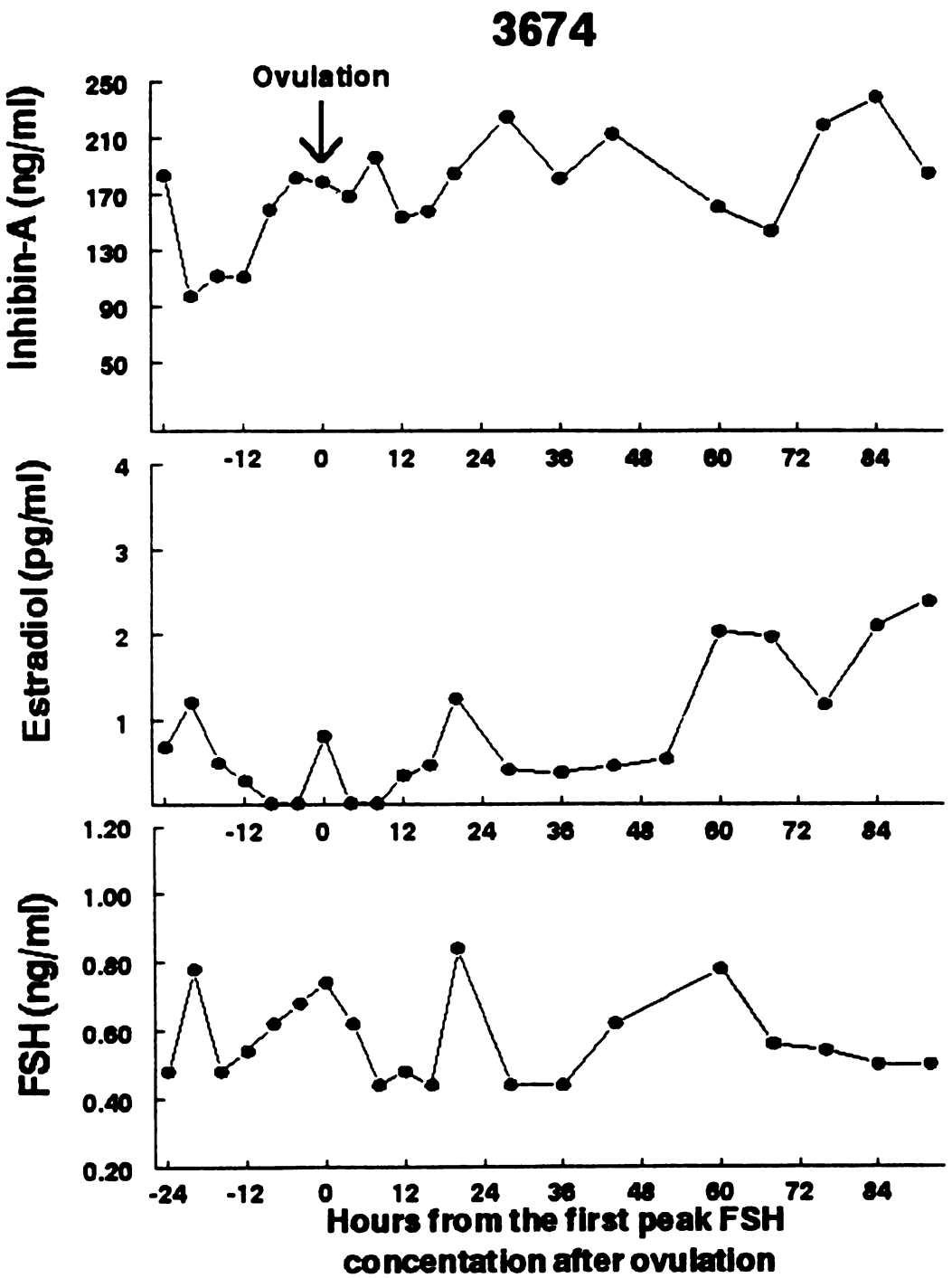


Figure 49. Alterations in total numbers of antral follicles (≥ 3mm) during the first wave of an estrous cycle of cow ID 3539.

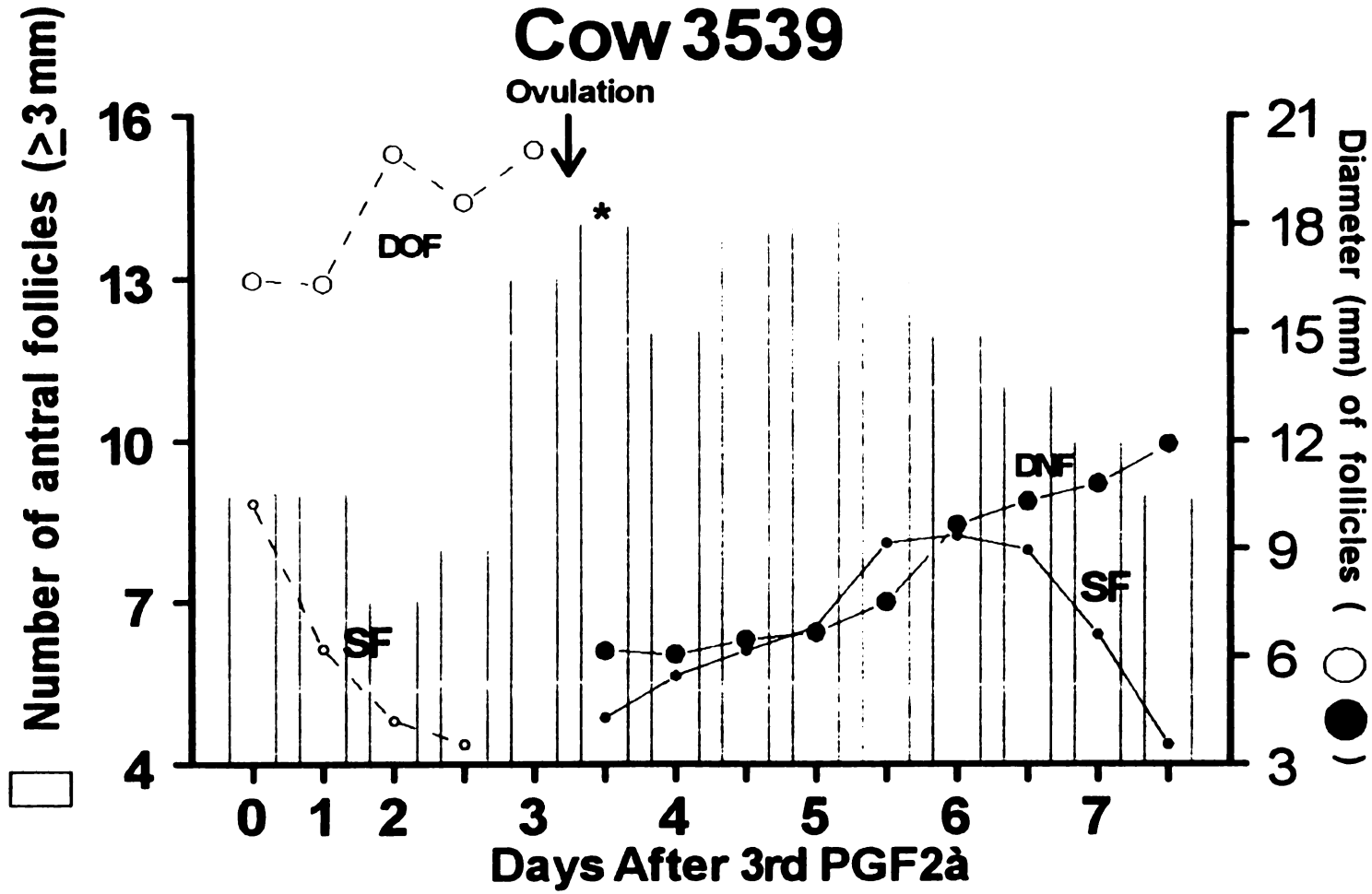


Figure 50. Inhibin-A, estradiol, and FSH profiles aligned to the first FSH surge for cow ID 3539.

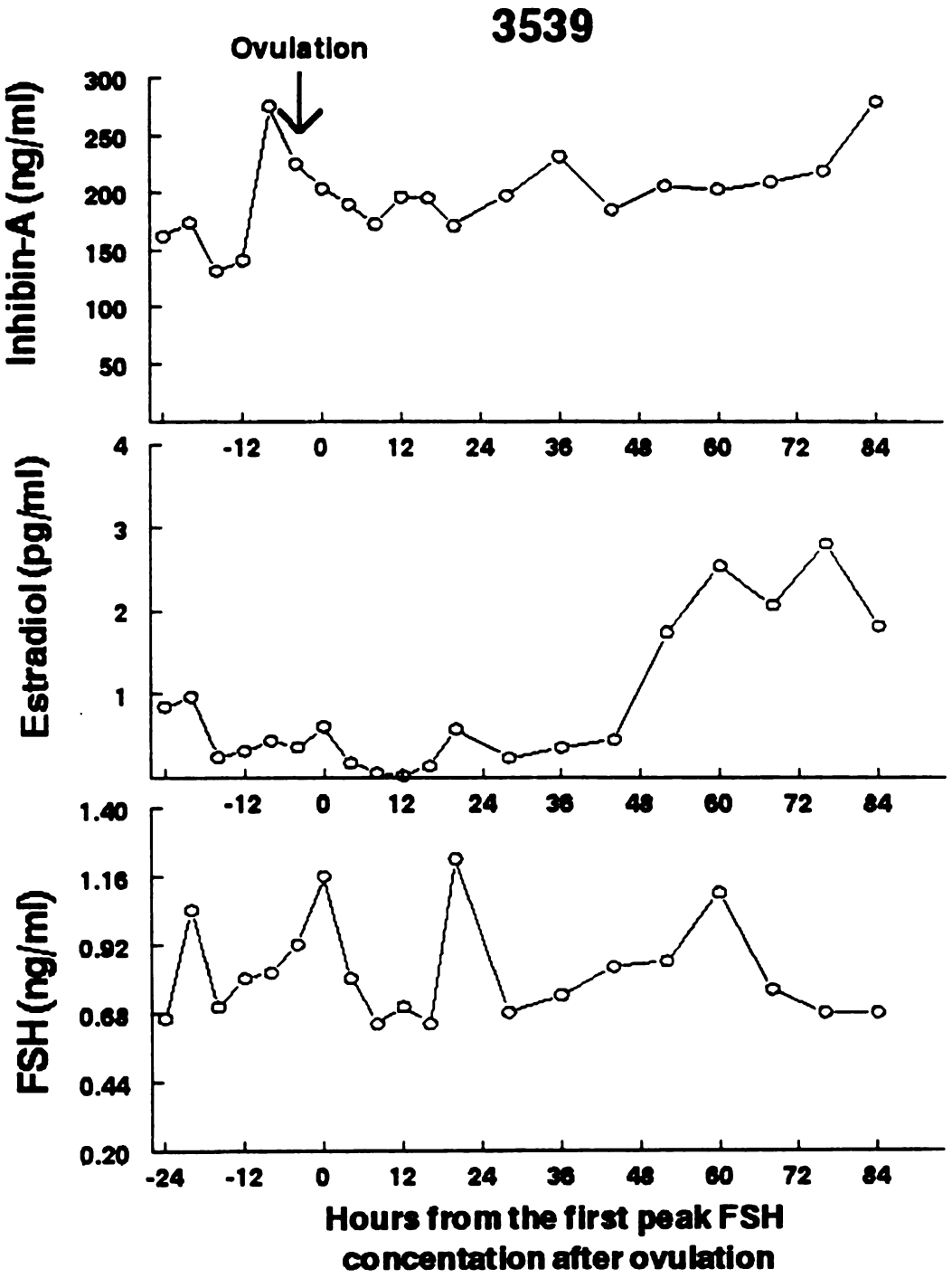


Figure 51. Alterations in total numbers of antral follicles (≥ 3mm) during the first wave of an estrous cycle of cow ID 3456.

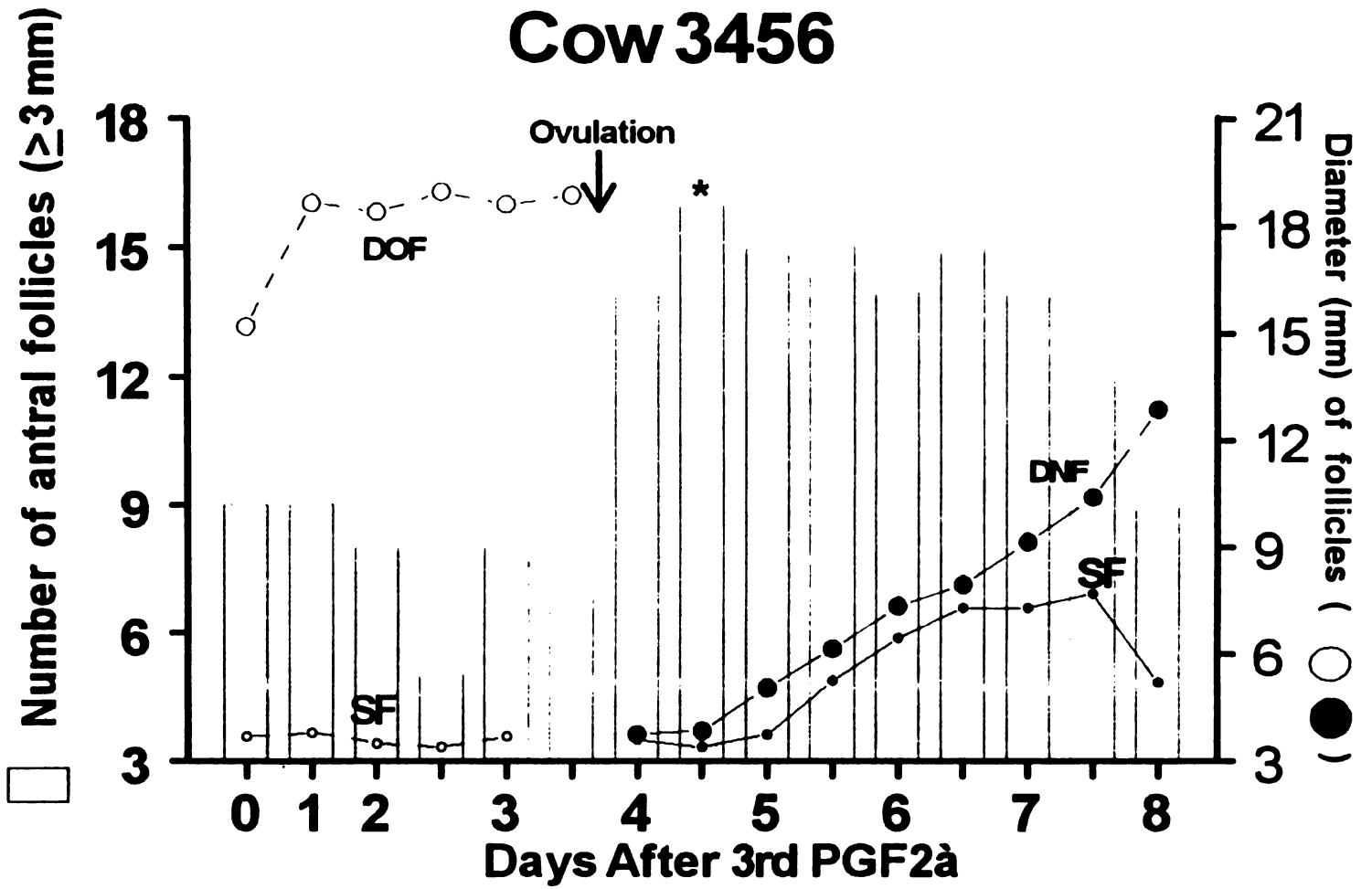


Figure 52. Inhibin-A, estradiol, and FSH profiles aligned to the first FSH surge for cow ID 3456.

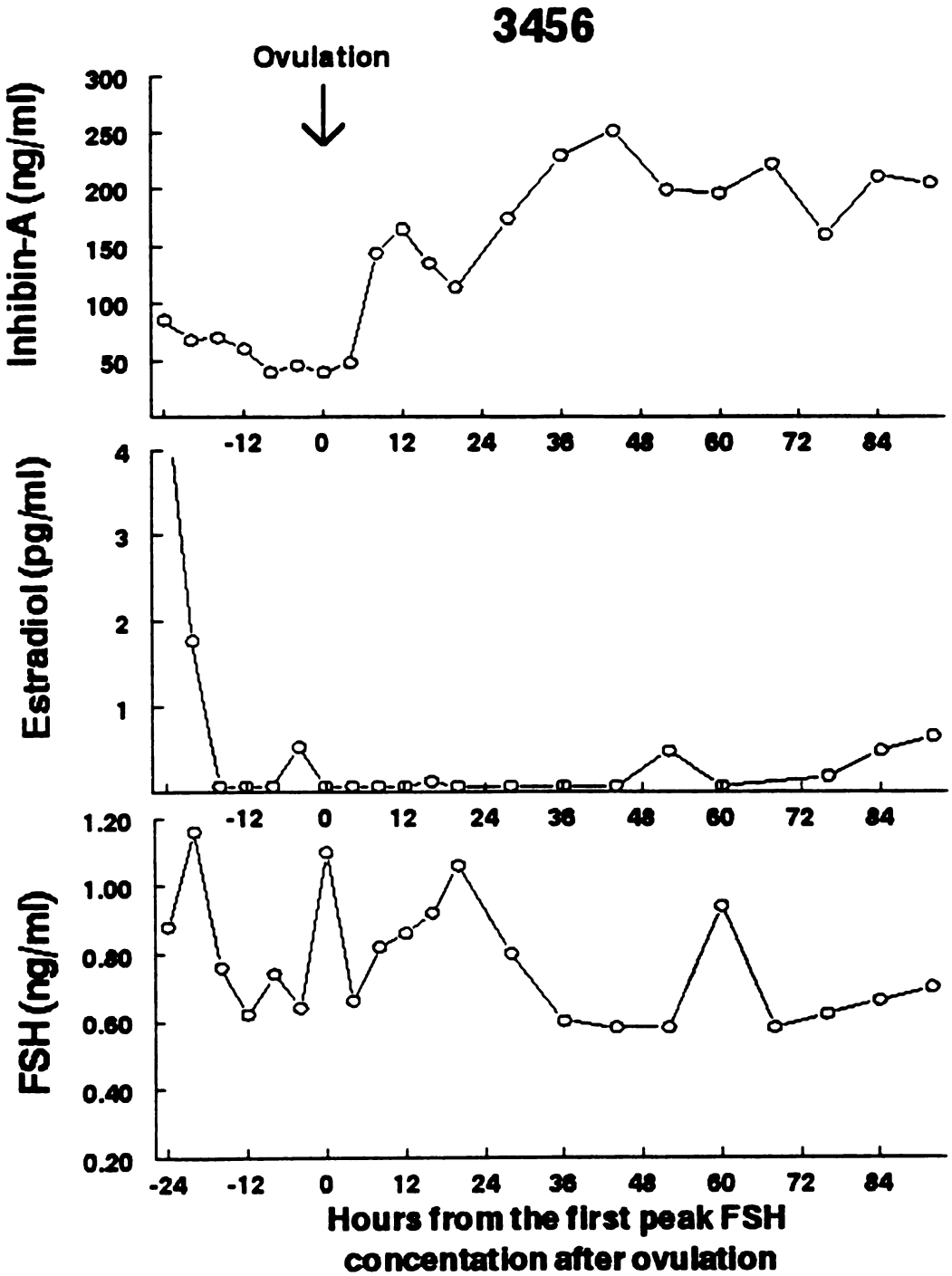


Figure 53. Alterations in total numbers of antral follicles (≥ 3mm) during the first wave of an estrous cycle of cow ID 3612.

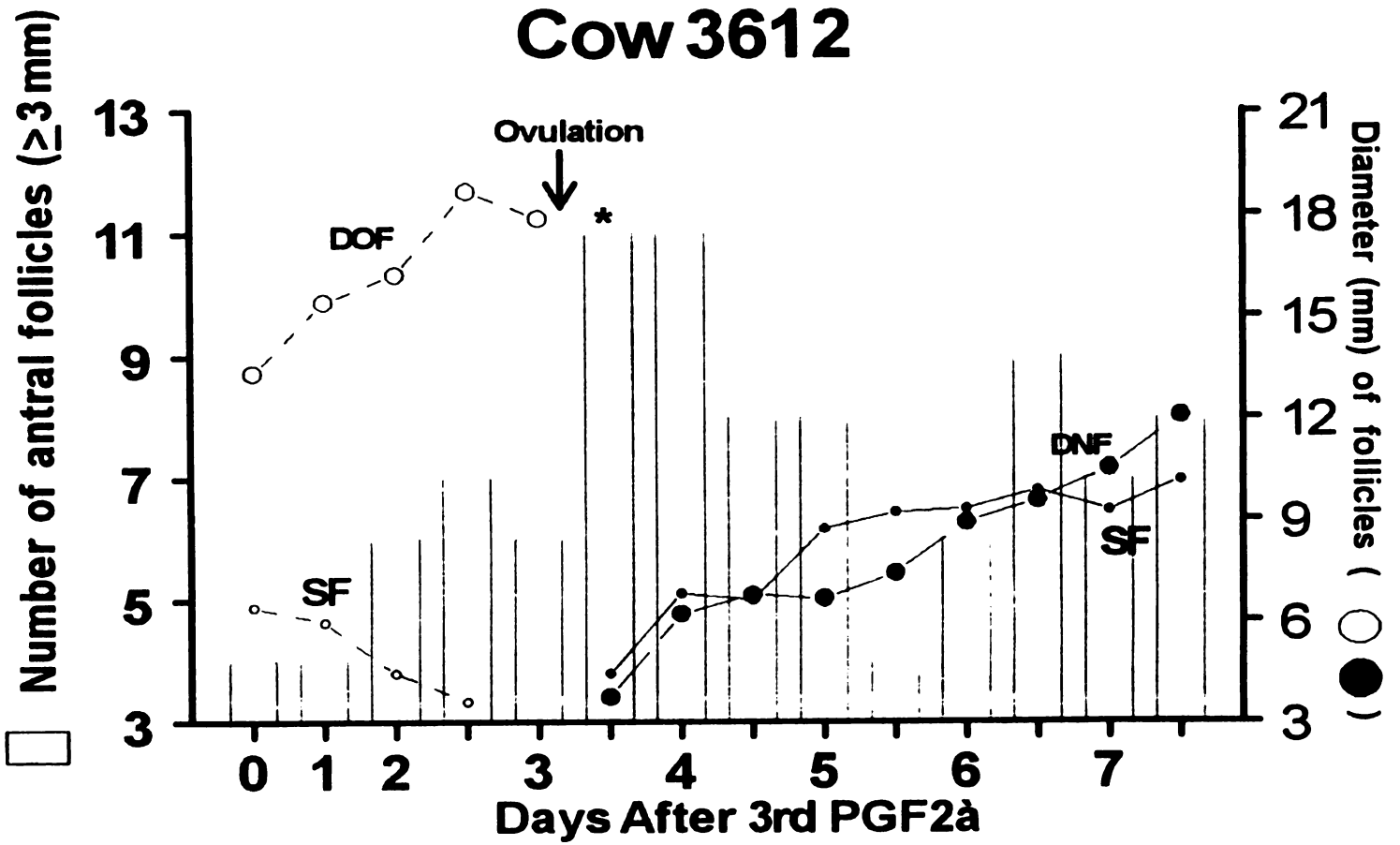


Figure 54. Inhibin-A, estradiol, and FSH profiles aligned to the first FSH surge for cow ID 3612.

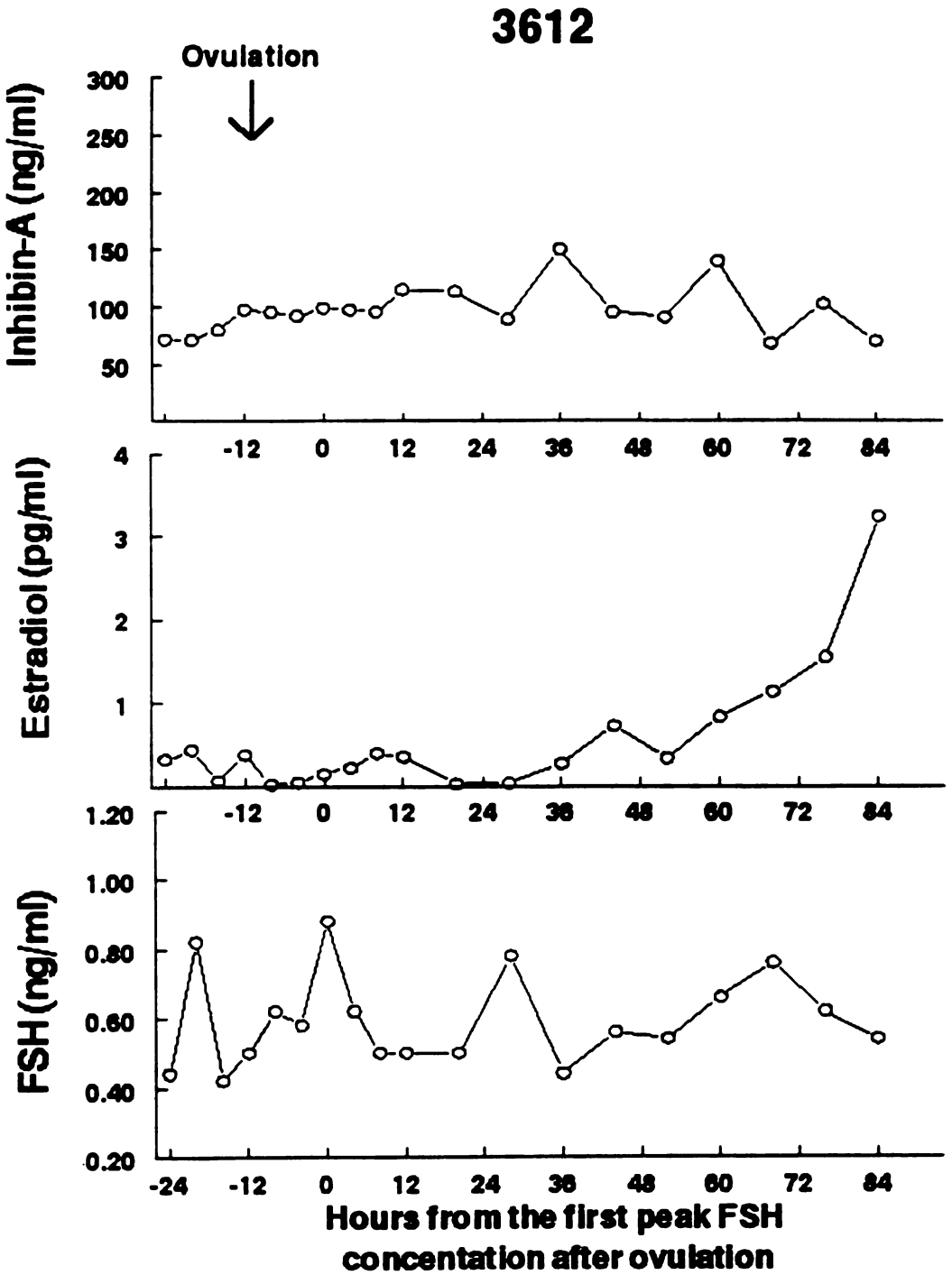
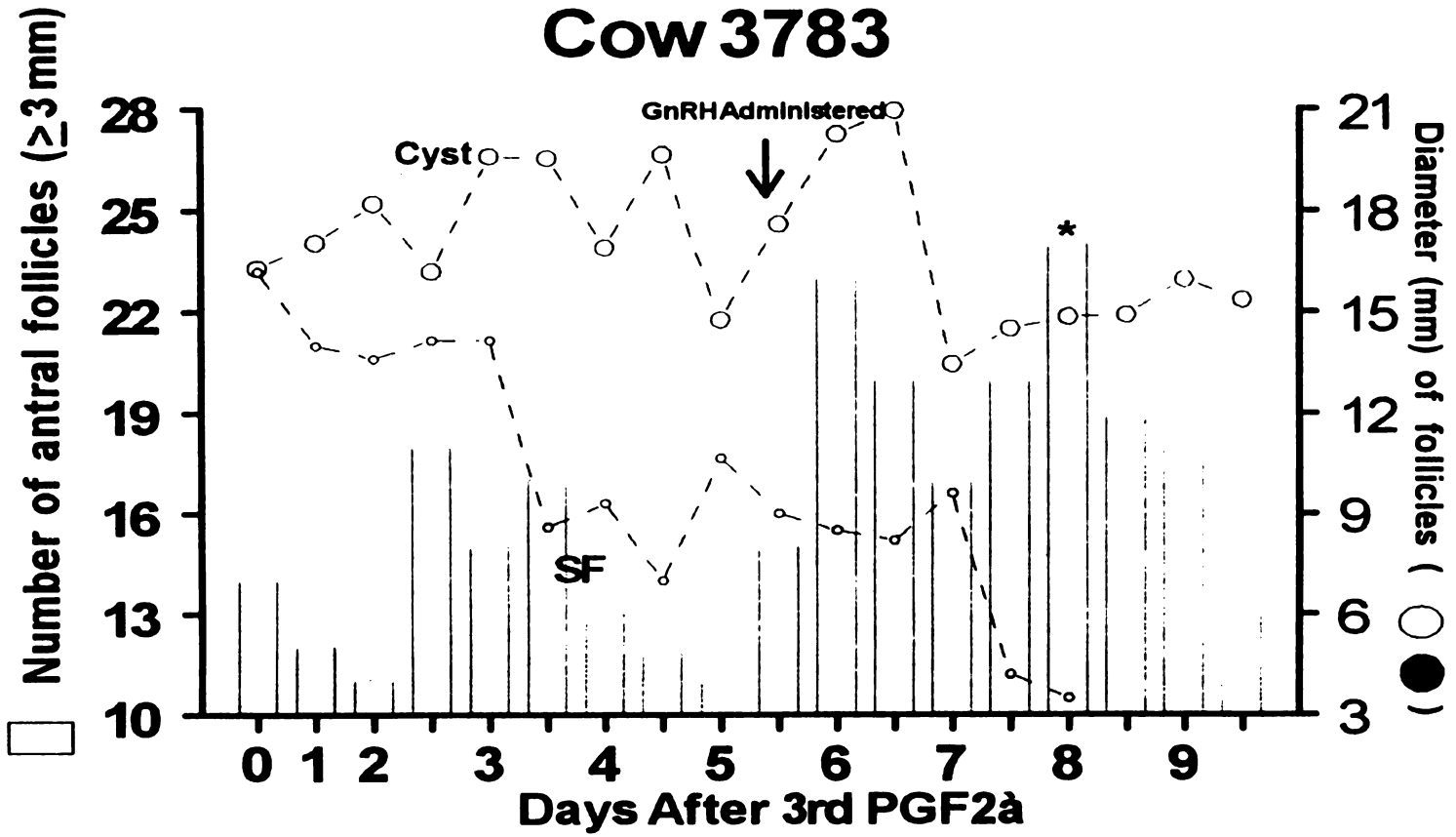


Figure 55. Alterations in total numbers of antral follicles (≥ 3mm) during the first wave of an estrous cycle of cow ID 3783.



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