OVARIAN AND ENDOCRINE ACTIVITY IN ANGUS, BRAHMAN AND SENEPOL COWS

By

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NOMENCLATURE

ANOVA	analysis of variance
BW	body weight
BCS	body condition score
CL	corpus luteum
FSH	follicle stimulating hormone
GH	growth hormone
GLM	general linear model
IGF	insulin-like growth factor
IGFBP	insulin-like growth factor binding protein
kg	kilogram
LH	luteinizing hormone
ml	milliliter
mm	millimeters
ng	nanograms
RIA	radioimmunoassay
PGF	prostaglandin
P4	progesterone

CHAPTER I

INTRODUCTION

Reproductive performance is one of the most critical factors affecting the profitability of beef cattle operations. Reproductive performance depends upon the ability of a cow to yield a viable calf every year. To achieve this, ovarian follicles must be able to undergo a growth and maturation process in order to provide an optimum environment to nurture an oocyte capable of being fertilized. In cattle, the process of growth and development of follicles, folliculogenesis, is not completely understood, but involves a complex network of hormones and growth factors. Understanding the sequence of events and factors involved in follicle development and ovulation will enhance our ability to manipulate reproductive parameters in order to achieve more efficient and profitable production. In subtropical regions, environmental effects on reproduction represent the main limiting factor in cattle production, since hot and humid conditions hinder reproductive efficiency of cattle. The ability of animals to reproduce while attempting to overcome hot and humid conditions depends on both their degree of environmental adaptation and their genetic capacity (Chenoweth, 1994). The introduction of breeds of cattle more adapted to subtropical conditions as purebreds or crossbreds has been suggested as a way to improve the efficiency of beef production in those environments.

Beef cattle production in subtropical environments is increasingly influenced by Brahman and Brahman-cross cattle. The introduction of Bos indicus cattle in the United States has had favorable effects related to a greater tolerance to internal and external parasites, better adaptability to high ambient temperatures and humidity, and the ability to use poor quality forages. Nonetheless, several traits such as longer gestation periods and lower fertility have hinder the reproductive performance of Bos indicus cattle (Warnick, 1963; Plasse, 1973). The introduction of Bos taurus genotype adapted to tropical environments would take advantage of the superior reproductive traits of Bos taurus together with the greater adaptation of Bos indicus to tropical environments. The search for new breeds of cattle with those characteristics would greatly improve the efficiency of beef cattle production in those environments.

CHAPTER II

REVIEW OF LITERATURE

OVARIAN FUNCTION IN CATTLE

One of the main functions performed by the ovary is folliculogenesis. Folliculogenesis involves the formation of mature (Graafian) follicles, able to ovulate, from a pool of non-growing (primordial) follicles (Spicer and Echternkamp, 1986). A primordial follicle consists of an oocyte surrounded by a single layer of granulosa cells. Secondary follicles form from these primordial follicles once the granulosa cells have undergone mitosis. Tertiary follicles develop from secondary follicles once an antrum is formed. In a Graafian follicle, a large tertiary follicle, the oocyte is surrounded by several layers of granulosa cells and an antrum full of follicular fluid (Marion et al., 1968). The growth of a follicle from the primordial stage to the antral stage is a continuous process which ends with ovulation (Hirshfield, 1991).

Ovarian follicular growth and development

Follicle growth and maturation involves a sequence of morphologic, physiologic and biochemical transformations of the components of the follicle such as the oocyte,

granulosa and thecal cells (Hirshfield, 1991). In this process the follicle gradually acquires a number of properties, each of which enables the follicle and its oocyte to grow and mature. The follicles that fail in acquiring those specific properties will regress in a process called atresia (Campbell et al., 1995). The growth of antral follicles (i.e. tertiary follicles) occurs through first, proliferation of granulosa cells, second, the increase in cytoplasmic and nuclear volumes of the oocyte (Lintern-Moore and Moore, 1979), and third, the accumulation of follicular fluid which leads to the formation of the antrum (Hirshfield, 1991). In cattle, folliculogenesis begins during fetal life with the formation of primordial follicles beginning around day 100 of gestation (Henricson and Rajakoski, 1959). Primordial follicles represent a pool of nongrowing follicles present in the ovary that will be progressively depleted throughout the reproductive life span of the female by either attrition or initiation of follicular growth (Erickson, 1996). The mechanisms by which some primordial follicles are selected to grow whereas others remain quiescent is unknown (Wandji et al., 1996).

Hodgen (1982) defined folliculogenesis as a three stage process: 1) recruitment is the first stage in which a pool of follicles begin to develop and become sensitive to gonadotropin stimulation; 2) selection occurs when a group of follicles from the recruited pool are selected to overcome atresia and survive to ovulate and 3) dominance is the mechanism by which the selected, potentially ovulatory follicle inhibits the growth of other follicles and dictates the course of events of the estrous/menstrual cycle. The three stages of folliculogenesis are controlled by several factors which include the intrafollicular steroids, growth factors, extraovarian factors, the hypothalamo-pituitary-ovarian feedback system and a complex network of relationships among them (Campbell et al., 1995).

Follicular waves

In cattle, follicle growth occurs in waves. A follicular wave is defined as the synchronous development of a group of large antral follicles (Knopf et al, 1989). During a follicular wave, one large antral follicle, the dominant follicle, is selected from a pool of growing antral follicles to become larger and "dominate" over the other follicles, through the inhibition of follicular growth (Sirois and Fortune, 1988). The dominant follicle will either regress by atresia (dominant non-ovulatory follicle) or ovulate (dominant ovulatory follicle) if the phase of dominance is followed by luteolysis (Sirois and Fortune, 1988).

The wave pattern of follicular growth in cattle was first suggested by Rajakoski (1960) , who reported two waves of follicular growth during the estrous cycle of cattle. One wave occurred between days 3 and 12 of the estrous cycle followed by a second wave between day 12 and the subsequent estrus following luteolysis. The two-wave hypothesis was supported by subsequent data of Matton and coworkers (1981) but challenged by Ireland and Roche (1983) who proposed the existence of not two but three waves of follicular growth in heifers. The introduction of ultrasound technology in 1984 created a major breakthrough in the study of follicular dynamics (Ginther et al., 1996). Ultrasound studies have revealed three different patterns of follicular growth consisting of either two, three or four waves (Ginther et al., 1989; Savio et al., 1988; Sirois and Fortune, 1988). In a two-wave pattern, which occurs at the frequency of about the 50 % of the population, the first follicular wave is identified on day 0 with the second wave occurring on day 10 of the estrous cycle. In a three-wave pattern, which occurs at a frequency of about 50 % of the population, the first wave is identified on day 0 to 4, the second wave on day 10 and the third wave on day 16 of the estrous cycle. In a four wave

pattern , which occurs at a frequency of less than 10 % of the population, the first wave is initiated on day 2, the second on day 8, the third on day 14 and the fourth on day 17 of the estrous cycle (Sirois and Fortune, 1988; Savio et al., 1988; Ginther et al., 1989; Knopf et al., 1989).

The number of follicular waves during an estrous cycle is regulated by the duration of the luteal phase. In heifers, estrous cycles with three follicular waves have longer luteal phases than cycles with two waves (Ginther et al., 1989) and cycles with 4 or 5 waves of follicular development have been reported when the luteal phase is prolonged by treating heifers with exogenous progesterone (Sirois and Fortune, 1990). However, a study by Murphy et al. (1991), indicated that well-fed heifers with predominantly two follicular waves had similar cycle lengths when compared with underfed heifers that had predominantly three follicular waves.

Follicular selection

The mechanisms by which a certain antral follicle becomes larger than the subordinate follicles and takes over the control of follicular dynamics is not completely understood. Initially, all the antral follicles from the growing pool have equal ability to become dominant (Ginther et al., 1996). Superovulation treatments with FSH can recruit several follicles capable of reaching dominance (Adams et al., 1993a) and the largest subordinate follicle turns into a dominant follicle if the dominant follicle is destroyed (Ko et al., 1991; Adams et al., 1993b). The dominant follicle becomes distinctly larger than the largest subordinate and deviates from the pattern of growth of the other (subordinate) follicles approximately 3 days into the start of a wave. Although the mechanisms by which

deviation is attained are unknown, it has been hypothesized that the dominant follicle may have a size advantage over the subordinates and therefore reaches a further stage of development earlier than the subordinates (Ginther et al., 1996). Also, an increase in the number of LH receptors in granulosa and thecal cells in the dominant follicle has been proposed as a major feature in the process of follicle selection (Bodensteiner et al., 1996; Xu et al., 1995; Stewart et al., 1996). In this model, an increase in LH receptor mRNA in granulosa cells between days 2 and 4 after the emergence of a wave and an increase in the binding of LH to both granulosa and thecal seems critical in the process of selection (Xu et al., 1995; Stewart et al., 1996). In further support of this latter suggestion, the number of hCG/LH binding sites in theca cells increase with increased diameter of estrogen-active follicles during days 3 to 7 of the bovine estrous cycle, whereas FSH binding sites in granulosa cells of estrogen-active follicles did not change during the same interval (Ireland and Roche, 1983).

Follicular dominance

The mechanism by which the dominant follicle suppresses the growth of the subordinate follicles is not completely understood. Apparently, a decrease in plasma concentrations of FSH occurs during luteolysis and the early follicular phase as the preovulatory follicle develops (Quirk and Fortune, 1986; Butler et al., 1983; Schallenberger et al., 1984). The dominant follicle, in a later stage of development than the subordinates, is able to overcome the decrease in FSH and keeps growing, whereas the growth and differentiation of the subordinate follicles is inhibited due to the lack of FSH (Ginther et al., 1996).

It has been also suggested that the dominant follicle produces specific inhibitors such as estradiol and inhibin which cause a decrease in FSH to levels that are not supportive of subordinate follicle development (Fortune, 1994; Ginther et al., 1996). In addition, an alternative mechanism of dominance has been proposed in which inhibitory factors from the dominant follicle other than inhibin are believed to directly suppress growth of subordinate follicles. Treatment of sheep (Campbell et al., 1991) and cattle (Law et al., 1992) with inhibin-free ovine or bovine follicular fluid inhibits ovarian hormone secretion and follicle development without altering peripheral concentrations of FSH.

Stewart et al. (1996) proposed that the ovarian insulin-IGF-I system may play a role in follicular dominance. Although large follicles have a greater number of granulosa cell IGF-I receptors than small follicles (Spicer et al., 1994), numbers of thecal and granulosa cell IGF-I receptors do not differ between subordinate and dominant follicles (Stewart et al., 1996). Number of binding sites for LH/hCG in thecal cells was greater in dominant follicles than in subordinate follicles during the late growing phase and increased first in theca then in granulosa cells; this increase in thecal LH receptors may be mediated by the action of IGF-I (Stewart et al., 1996). Similarly, IGFBPs may also play a role in follicular dominance by regulating the bioavailability of IGF-I in the follicle (Stewart et al., 1996). In cycling dairy cows, early dominant follicles had less IGFBP activity along with an increase in the number of thecal LH/hCG binding sites (Stewart et al., 1996). In the late dominant follicle, an increase in IGFBPs may block the stimulatory actions of IGF-I and lead to atresia (Stewart et al., 1996). In cattle, amounts of IGFBPs other than IGFBP-3 are much lower in large estrogen-active follicles than in small (< 5 mm), medium

(5-7 mm), or large atretic (≥ 8 mm) follicles (Echternkamp et al., 1994; Funston et al., 1996).

Role of gonadotropins

Gonadotropins, FSH and LH, secreted by the hypophysis, play a major role in regulating follicular growth and development. Gonadotropins stimulate ovarian steroidogenesis in granulosa and theca cells through the activation of cAMP. The result is an increase in the concentrations of steroids, especially estradiol-17β, in plasma and in the follicular fluid of antral follicles. Enhanced ability to produce estradiol is characteristic of the selected follicles, and is essential in the process of differentiation and dominance (Fortune, 1994). Follicular estradiol secretion is dependent on the action of FSH and LH on granulosa and thecal cells, respectively. The actions of gonadotropins in granulosa and thecal cells have been extensively reviewed (Hillier et al., 1995).

Follicular development is regulated by inhibitory and stimulatory ovarian factors that act through the regulation of gonadotropin secretion and responsiveness of the follicle to gonadotropins (Ireland, 1987; Findlay, 1994). The inhibition of the dominant follicle upon the subordinate follicles is exerted via systemic endocrine channels rather than in an autocrine or paracrine fashion, since no intraovarian relationships between follicles were detected (Ginther et al., 1989). In a previous study (Miller et al., 1979), treatment of heifers with a proteinaceous fraction of bovine follicular fluid, presumably high in inhibin concent, suppressed follicular activity and caused a delayed time to estrus. In support of this early study, inhibin, a glycoprotein present in bovine follicular fluid (Robertson et al., 1985), selectively suppressed the synthesis and secretion of FSH in heifers (Beard et al., 1990; Kaneko et al., 1993) and lambs (Tortonese and Gomez-Brunet, 1996). In addition,

the dominant follicle secretes factors other than inhibin that can suppress follicular growth, because the administration of inhibin-free follicular fluid in heifers caused a decrease in follicular growth (Law et al., 1992). Conversely, further studies indicated that treatments with follicular fluid suppressed concentrations of FSH in plasma without altering plasma LH in ovariectomized (Ireland et al., 1983) and intact heifers (Quirk and Fortune, 1986). Consistent with these latter observations, immunization of cows against inhibin increased FSH concentrations (Kaneko et al., 1993; Glencross et al., 1994).

It is hypothesized that increased concentrations of FSH in plasma is the signal required for follicular recruitment in the early phases of the estrous cycle, since there is a temporal relationship between increases in circulating FSH and the recruitment of follicles (Ginther et al., 1996). Several evidences support this hypothesis. In rats, a secondary surge of FSH occurs on the day of estrus just before the recruitment of the next cohort of ovulatory follicles (Smith et al., 1975). In primates, basal plasma concentrations of FSH are slightly greater at the beginning of the follicular phase than during the luteal or late follicular phases (Abraham et al., 1972; Goodman et al., 1977) and an increase in FSH on the day of ovulation precedes the first follicular wave of the estrous cycle in cattle (Dobson, 1978; Walters and Schallenberger, 1984). More recently, a functional relationship between surges in circulating concentrations of FSH and the emergence of follicular waves was observed in prepubertal heifers (Adams et al., 1994), postpubertal heifers (Adams et al., 1992b) and pregnant heifers (Ginther et al., 1996). A significant surge of FSH was detected 1 or 2 days prior to the emergence of a follicular wave, and advancing or delaying the surge, using follicle cautery or follicular fluid treatment, was associated with an equivalent advance or delay in wave emergence (Adams et al., 1992a;

1992b). Similar relationships have been shown in ewes (Ginther et al., 1995) and mares (Bergfelt and Ginther, 1993). Existence of a correlation between plasma FSH surges and follicular recruitment is supported by several facts. Alterations in the surges of FSH resulted in changes in the patterns and/or number of recruited follicles. Follicular development is inhibited in hypophysectomized rats and by short term suppressions of FSH in blood (Nakano et al., 1975). FSH alone restores antral follicle development in rats (Richards, 1980). Abolishing the secondary surge of FSH by injections of follicular fluid containing inhibin prevents the recruitment of the ovulatory cohort of the next cycle in rats (Grady et al., 1982) and delays the first follicular wave of the cycle in cattle (Turzillo and Fortune, 1993).

Although FSH seems to be important during the initial stages of follicular development, LH apparently plays a major role in the later stages of folliculogenesis. In cattle, small increases in basal LH, and increases in LH pulse frequency follows the decrease in plasma progesterone concentrations at luteal regression (Spicer et al., 1981; Walters and Schallenberger, 1984). Elevation in circulating LH results in a greater response of the thecal cells in terms of androgen production and a subsequent increase in aromatization capacity of the granulosa cells (Fortune, 1994). Several studies in which plasma progesterone was artificially maintained at low levels for prolonged periods of time confirm the role of LH in later stages of follicular development. In heifers treated with low levels of progesterone, LH pulse frequency increased to follicular phase levels (Robertson et al., 1989; Stock and Fortune, 1993), growth of the dominant follicle continued in a linear fashion, and plasma estradiol was elevated (Sirois and Fortune, 1990; Stock and Fortune, 1993).

It has been proposed that at a certain stage of follicular development there is a shift in gonadotropin dependence from FSH to LH (Campbell et al., 1995; Ginther et al., 1996). As a result, the large dominant follicle becomes highly dependent on LH for support and cessation of high frequency LH pulses will lead to its rapid atresia. This change of gonadotropic requirement from FSH to LH may explain the mechanism by which the preovulatory follicle overcomes the decrease in FSH that occurs at the onset of the follicular phase following luteal regression (Ginther et al., 1996; Campbell et al., 1995). This hypothesis is further emphasized by the extension of the lifespan of the dominant follicle by increasing LH pulse frequency (Fortune, 1994; Savio et al., 1993). In addition, chronic treatment of cattle with a GnRH agonist suppressed the pulsatile secretion of LH, and the largest follicle did not grow beyond 7 to 9 mm (Gong et al., 1995).

Luteal growth and development

Corpora lutea are formed following ovulation from the remaining follicular cells of the ovulatory follicle. The corpus luteum (CL) consists of several types of cells with distinct morphological, endocrinological and biochemical characteristics (Fields and Fields, 1996). Cell types that have been identified in the CL of domestic ruminants include small luteal cells, large luteal cells, fibroblasts, endothelial cells, and macrophages (Farin et al., 1986; Fields and Fields, 1996). In cattle, granulosa cells differentiate into large luteal cells and thecal cells differentiate into small luteal cells (Donaldson and Hansel, 1965; O'Shea, 1989).

Luteinization is a complex process which transforms the preovulatory follicle into

a highly vascularized tissue capable of secreting large amounts of progesterone. The preovulatory surge of FSH and LH produces a series of morphological, endocrinological and biochemical changes in granulosa and thecal cells (Priedkalns et al., 1968; McClellan et al., 1975; Jablonka-Shariff et al., 1993; Zheng et al, 1994). In the process of formation and development of the CL, various cell types undergo hyperplasia, hypertrophy and (or) migration (O'Shea et al., 1980). The hypertrophy of the luteal cells accounts for most of the mass increase during CL development, however, small steroidogenic luteal cells, endothelial cells and fibroblasts proliferate and may also contribute to the growth of the CL (Smith et al., 1994; Zheng et al., 1994). Apparently, large luteal cells proliferate during the early stages of luteal development and small luteal and non-steroidogenic luteal cells continue to proliferate until luteolysis is initiated (Jablonka-Shariff et al., 1993; Priedkalns et al., 1968; McClellan et al., 1975).

The growth and development of the CL follows a sequential pattern during the estrous cycle. In cattle, the period of growth is slightly longer than half the estrous cycle. Specifically, the weight and size of the CL increase rapidly between days 0 and 12 of the estrous cycle and remain relatively constant until day 14 to 16, when CL regression begins; if fertilization does not occur, the CL regresses due to the secretion of $PGF_{2\alpha}$ from the uterus (Spicer et al., 1981; Pierson and Ginther, 1984; Kastelic et al., 1990; Zheng et al., 1994).

The primary function of the CL is the secretion of progesterone. Progesterone plays a major role in preparing and maintaining a uterine environment favorable for pregnancy to occur (Fields and Fields, 1996). In cattle, small and large luteal cells are responsible for the production of progesterone, however these types of cells are

differentially regulated (Urseley and Leymarie, 1979; Hansel et al., 1987). Progesterone production by the large luteal cells is relatively independent of LH action, since in vitro they showed little responsiveness to LH stimulation (Hoyer and Niswender 1985; Hansel et al., 1991) but the small luteal cells respond to LH stimulation. In cattle, basal secretion of progesterone in vitro was less in small luteal cells than in large cells (Urseley and Leymarie, 1979; Hansel et al., 1991). Large luteal cells which represent 40% of the luteal volume, are the major producers of luteal progesterone (80%) as compared to small luteal cells which are responsible for the remaining 20% (Niswender et al., 1985). Progesterone production by both cell types is dependent on lipoproteins delivered by the profuse luteal blood supply (Wiltbank, 1994). Although LH action is critical in the early stages of luteinization, growth and development of the CL, progesterone production by the mid-cycle CL of ruminants is relatively independent of LH stimulation (Wiltbank, 1994).

During the estrous cycle in cattle, systemic progesterone concentrations are minimal during the first 3 days of the cycle. Progesterone starts to increase during the 4th-6th day of the estrous cycle, reaching a maximum of 3 to 6 ng/ml between the 11th and 18th day. Following luteolysis, plasma progesterone concentrations drop rapidly 72 to 96 hr before the onset of the next estrus, which occurs 48 to 72 hr after the plasma concentrations of progesterone falls to less than 0.5 ng/ml (Robertson, 1972; Wettemann et al., 1972).

The size of the CL and its capacity to produce progesterone are correlated for most of the estrous cycle. In postpartum cows, CL diameter, determined by ultrasonography is positively correlated with milk progesterone concentrations (Sprecher et al., 1989). Similarly, in nulliparous heifers, luteal tissue area (determined by

ultrasonography) and plasma progesterone concentration were highly correlated (Kastelic et al., 1990). In this study, progesterone concentrations in the systemic circulation and CL size increased at a similar rate during luteal growth (days 2, 5 and 8), but during luteal regression, luteal tissue area decreased more slowly than plasma progesterone concentration. In addition, Ribadu et al., (1994) found a correlation (r = 0.85) between the diameter of the CL and progesterone concentrations in lactating dairy cows, however, the correlation was absent during the last days of the cycle, when the diameter of the CL was the same as mid-luteal values but it was functionally inactive. Similarly Rajamahendran and Taylor (1990) found a strong correlation between maximum diameter of the CL and peak plasma progesterone concentrations in the second postpartum estrous cycle in dairy cows.

INSULIN LIKE GROWTH FACTORS I AND II AND THEIR BINDING PROTEINS IN DOMESTIC ANIMALS

Plasma concentrations of IGF-I and -II during the estrous cycle

Secretion of IGF-I during estrous cycles differs among species. Serum concentrations of IGF-I in ewes change significantly during the estrous cycle, with greater concentrations of IGF-I 1 to 2 days after estrus than 5 to 6 days after estrus, and greater concentrations on days 13 to 14 than on days 7 to 8 of the cycle (Spicer and Zavy, 1992; Spicer et al., 1993b). Similarly, Leeuwenberg et al. (1996) found a significant increase in IGF-I concentrations in plasma from day -1 to day 1 (day 0 = day of estrus) of the estrous

cycle in ewes. In mares (Spicer et al., 1991), plasma concentrations of IGF-I do not differ between early (day 1 or 2) and late estrus (day 3 to 6). In gilts, systemic IGF-I concentrations did not differ throughout the estrous cycle (Wiseman et al., 1992; Whitley et al., 1995). In cattle, serum concentrations of IGF-I did not change during the first ten days of the estrous cycle (Stewart et al., 1996). In contrast, Rutter and co-workers (1989) reported that circulating IGF-I increased approximately 2 days before ovulation in postpartum cows. In another study, serum concentrations of IGF-I were less on day 18 than day 10 or 15 postestrus in both pregnant and non-pregnant cows (Spicer and Geisert, 1992).

The sparse literature published to date suggests that systemic levels of IGF-II seem to remain constant throughout the estrous cycle. In cyclic dairy cows, serum concentrations of IGF-II did not differ between day 5 and 10 of the estrous cycle (Stewart et al., 1996). In pregnant lactating cows, serum concentrations of IGF-II were not different between early and late lactation (Vicini et al., 1991). In mature gilts, plasma levels of IGF-II were not different throughout the estrous cycle (Wiseman et al., 1992).

In summary, IGF-I concentrations during the estrous cycle seem to remain constant in swine and mares, whereas some variations during the estrous cycle have been observed in ruminants (cattle and sheep).

Actions of IGF-I and IGF-II in the ovary

Insulin-like growth factor-I is found in many tissues in the body, including the ovary of several domestic species (Hammond et al., 1985; Spicer et al., 1992; 1988; Spicer and Zavy, 1992; Spicer et al., 1991; 1995). Within the ovary, IGF-I is produced in granulosa, thecal, stromal, and luteal cells (Murphy et al., 1987; Einspanier et al., 1990; Samaras et al., 1994; Spicer et al., 1993a; 1995), where it is thought to act as an autocrine or paracrine regulator of ovarian function.

IGF-I is involved in follicular growth and development. In pigs, cattle and sheep, IGF-I also has a stimulatory effect on granulosa cell proliferation and DNA synthesis in vitro (Spicer et al., 1993a; Baranao and Hammond, 1984; Dorrington et al., 1987; Monniaux and Pisselet, 1992; Zhang and Bagnell 1993; Gong et al., 1993c). In addition, follicular fluid concentrations of IGF-I in cattle are positively correlated with follicular diameter (Spicer et al., 1988; Echternkamp et al., 1990).

IGF-I also has an effect on ovarian steroidogenesis. In cattle, swine and sheep, IGF-I has stimulatory effects on progesterone production by granulosa, thecal and luteal cells (Baranao and Hammond, 1984; Caubo et al., 1989; McArdle et al., 1991; Spicer et al., 1993a; Sauerwein et al., 1992; Monniaux and Pisselet, 1992; Spicer et al., 1995). IGF-I has a stimulatory effect on LH-induced thecal cell androgen biosynthesis in pigs (Caubo et al., 1989) and cattle (Stewart et al., 1995). In pigs, IGF-I stimulates estradiol production (Maruo et al., 1988; Howard and Ford, 1994) and follicular cell of IGF-I mRNA levels and follicular fluid estradiol concentrations were correlated positively ($r = 0.69$) (Samaras et al., 1993). In cattle, IGF-I has little or no effect on basal and FSH-induced estradiol production by bovine granulosa cells from small and large follicles

(Spicer et al., 1993; Gong et al., 1994), whereas, IGF-I inhibits insulin-stimulated estradiol production by granulosa cells of small and large bovine follicles (Spicer et al., 1994). In addition, concentrations of IGF-I and estradiol in the follicular fluid of cattle can be either correlated negatively (Spicer et al., 1988) or positively (Echternkamp et al., 1990; Spicer and Enright, 1991) suggesting that changes in the intrafollicular concentrations of IGF-I are not consistently regulating estradiol production by bovine follicles.

IGFBPs plasma concentrations during the estrous cycle

Insulin-like growth factor binding proteins (IGFBPs) are soluble carrier proteins present in plasma, follicular fluid, and in other body fluids (Rechler, 1993). Six different species of IGFBPs have been identified: IGFBP -1, -2, -3, -4, -5, -6. The presence of IGFBPs in the systemic circulation has been reported in porcine (McCusker et al., 1989), bovine (Conover, 1990), ovine (Spicer et al., 1995) and equine (Prosser and McLaren., 1992) species. Systemic concentrations of IGFBPs do not change during the estrous cycle. Specifically, in mature beef cows, at four different stages of the estrous cycle, serum binding activity of IGFBP-3 (40-44 kDa), IGFBP-2 (34 kDa), IGFBP-5 (30 kDa) and a 28 kDa and 24 kDa IGFBP did not change with the stage of estrous cycle (Funston et al., 1995). Likewise, in gilts, serum concentrations of IGFBP 1-4 did not differ between follicular (days 17 to 19) and luteal phase (days 7 to 9) (Whitley et al., 1995).

Actions of IGFBPs in the ovary

IGFBPs are produced in vitro by several cell types including human (Giudice et al., 1990), rat (Nakatani et al., 1991) and porcine granulosa cells (Mondschein et al., 1990).

In the systemic circulation, IGFBPs prolong the half-life of IGF-I and regulate the endocrine actions of IGFs. In cell lines and tissues, IGFBPs regulate the local actions of the IGFs in an autocrine and/or paracrine fashion (Clemmons and Underwood, 1991). Presumably, IGFBPs function in the ovary to exert a regulatory role on the biological activity of IGF-I. Several in vitro studies with human and rat granulosa cells indicate IGFBPs have an inhibitory effect of ovarian in estrogen production (reviewed in Spicer and Echternkamp, 1995). In addition, follicular fluid progesterone is negatively correlated ($r = -.70$) with follicular wall IGFBP-2 mRNA expression in cyclic gilts (Samaras et al., 1993), and estradiol levels in ovine and bovine follicles were negatively correlated ($r = -.57$ to $-.74$) with follicular fluid IGFBP-2 (Spicer et al., 1995; Echternkamp et al., 1994). IGFBP-3 inhibits bovine thecal cell progesterone and androstenedione production in vitro (Spicer et al., 1997). Intrabursal administration of IGFBP-3 reduces the number of ovulations in immature rats treated with hCG (Bicsak et al., 1991). Thus, it appears that IGFBP-2 and -3 have a primarily inhibitory role in ovarian function.

Intrafollicular concentrations of IGFBP and activity decrease with follicular development and maturation. In cyclic gilts, follicular wall IGFBP-2 mRNA is negatively correlated ($r = -.70$) with the diameter of the follicle and with day of follicular development ($r = -.71$) (Samaras et al., 1993). In weaned sows, levels of IGFBP-2 decrease with advancing follicular development whereas IGFBP-3 levels do not change (Howard and Ford, 1992). In cattle, follicular fluid IGFBP-2 and follicular diameter are correlated negatively ($r = -.81$) (Echternkamp et al., 1994). Content of IGFBP-2 in follicular fluid and binding activity was lower in large than in small ovine follicles (Monget et al., 1993; Spicer et al., 1995).

Follicular atresia is correlated with an increase in IGFBP activity. In ewes, atresia of follicles < 2 mm is associated with an elevation in IGFBP-like activity within the granulosa cells and follicular antrum (Monget et al., 1989). Atretic follicles contain more IGFBP-2 activity than healthy follicles in sheep (Monget et al., 1993; Besnard et al., 1996), cattle (Echternkamp et al., 1994), and swine (Guthrie et al., 1995).

GROWTH HORMONE IN FEMALE CATTLE

Growth hormone plasma concentrations during the estrous cycle

Growth hormone (GH) secretion in cattle is pulsatile (Gluckman and Breier, 1987). Clusters of two to three high amplitude GH pulses occur in plasma at approximately 6-h intervals, separated by periods of declining basal GH levels (Moseley et al., 1982). In steers monitored for 24 h, GH patterns consist of several secretory bursts separated into three or four episodes by interlining periods of decreasing levels with an average of .7 pulses per hour (Wheaton et al., 1986).

Whether ovarian steroids influence secretion of GH in female cattle is controversial. Ovariectomy did not affect mean plasma GH concentrations or pulse frequency in cyclic heifers (Enright et al., 1994). Also, Beck et al. (1976) showed that mean serum GH concentrations in heifers did not change over a 6 day period after ovariectomy in heifers treated with 17β-estradiol implants. In contrast, serum GH was greater during estrus than during the luteal phase of the estrous cycle of lactating cows (Koprowski and Tucker, 1973). In another study, mean GH concentrations in serum and

GH pulse amplitude in heifers were greater one week before puberty than at 3 weeks before puberty (Yelich et al., 1996).

Growth hormone actions in the ovary

The presence of GH receptors on the ovary of the cow (Lucy et al., 1993a; Vandehaar et al., 1995; Kirby et al., 1996) suggests possible direct effects of GH on ovarian function in addition to indirect effects via stimulation of hepatic IGF-I production. In vivo, GH increases the incidence of twin births in dairy cows (Cole et al., 1991; Esteban et al., 1994), and increases the ovulation rate in superovulated beef heifers (Gong et al., 1991; Herrler et al., 1994). Moreover, exogenous GH increased the number of follicles (6 to 15 mm), and increased the size of the second largest ovarian follicle (De La Sota et al., 1993). On day 17 , cows treated with GH had more large follicles (10 to 15 mm) than non-treated cows (Lucy et al., 1995). In Holstein heifers, the emergence of the second follicular wave occurred earlier, the number of follicles ≥ 10 mm was greater, and the largest follicle on the second wave was smaller in GH-treated than in non-treated cows (Lucy et al., 1994). Thus, in vivo evidence indicates that GH may increase follicular growth in cattle, but whether these effects are direct or indirect via increased IGF-I secretion is unclear.

In vitro, GH weakly stimulated proliferation of bovine granulosa cells obtained from small follicles (1 to 5 mm) in the presence of insulin (Langhout et al., 1991). This increase in cell proliferation is not likely due to an increase in IGF-I production by granulosa cells because GH has no effect on IGF-I production by bovine granulosa cells (Spicer et al., 1993a). However, in cattle, GH had no effect on DNA synthesis of bovine

granulosa cells from small and medium-sized follicles, but inhibited incorporation of [^{3}H]-thymidine into granulosa cells from large follicles in a dose-dependent manner (Gong et al., 1993c). Spicer and Stewart (1996) found a weak inhibitory effect of GH in vitro on the proliferation of granulosa and thecal cells from large follicles. Collectively, effects of GH on follicular cell proliferation appears minor and varies with size of the follicle. Thus, it seems that the increase in the numbers of follicles induced by exogenous GH in vivo is not due to direct stimulatory effects of GH on follicular cell proliferation.

In addition to its effects in follicular growth and development, GH may regulate ovarian steroidogenesis. In vivo, GH treatment can either have no effect (Gong et al., 1991; Schemm et al., 1990), decrease (Lucy et al., 1994) or increase (Gong et al., 1993b; Lucy et al., 1993b) estradiol concentrations in plasma of cattle. Heifers treated with GH had larger CL and secreted more progesterone during the early (≤ day 10) and late (days 15 to 21) phases of the estrous cycle, but no changes in the ovulation rate were detected (Lucy et al., 1994). In Holstein cows, total progesterone area and progesterone mean concentration were increased in GH-treated cows during the first two cycles post-treatment and during pregnancy (Gallo and Elliot, 1991). Whether effects of GH on follicular and luteal steroidogenesis are direct or indirect is unclear. In support of direct effect of GH, in vitro studies have indicated that GH weakly stimulates progesterone secretion by bovine granulosa cells in the presence (but not in the absence) of insulin (Langhout et al., 1991) in vitro. However, GH treatment did not affect FSH-induced progesterone production by granulosa cells from small and large follicles, nor did it affect LH-induced progesterone production by thecal cells (Langhout et al., 1991; Spicer and Stewart, 1996). Spicer and Stewart (1996) also reported that GH inhibited FSH-

stimulated estradiol secretion by granulosa cells from small and large follicles. Moreover, in thecal cells, in vitro treatment with GH had a stimulatory effect on androstenedione production by bovine thecal cells responsive to LH whereas GH inhibited androstenedione production in thecal cells that did not respond to LH (Spicer and Stewart, 1996). Thus, in vitro studies indicate that GH has no effect on progesterone production by granulosa and thecal cells but GH may inhibit granulosa cell estradiol production and thecal androstenedione production.

INSULIN IN DOMESTIC ANIMALS

Concentrations of insulin in plasma during the estrous cycle

Concentrations of insulin in plasma change with the stage of the estrous cycle in cattle. In heifers, Reimers et al. (1982) found higher basal serum insulin concentrations during estrus than during diestrus. However, in another study pretreatment serum insulin concentrations were unaffected by the stage of the estrous cycle in lean and obese heifers (McCann and Reimers, 1985). Differences in time of day of blood collection and length of fasting between the two studies may explain the discrepancy. Also, estrus in obese heifers enhanced the acute insulin response to glucose and antagonized the glucoregulatory effect of insulin (McCann and Reimers, 1985). Serum basal concentrations of insulin and glucose were greater during estrus than during diestrus in lean heifers, and this increase in insulin concentrations was greater in obese heifers (McCann and Reimers, 1986).

Insulin actions in the ovary

Insulin has been proposed as a regulator of ovarian function in several species including cattle (Adashi et al., 1985; Jia et al., 1986; Poretsky & Kalin, 1987; Savion et al., 1981; Saumande et al., 1991). Since Hammond et al. (1985) identified insulin receptors in the porcine granulosa cells, specific receptors for insulin and its gene expression have been found in several types of ovarian cells from various species including granulosa cells of pigs, cattle, rats and humans (Baranao and Hammond, 1984; Spicer et al., 1993a; Spicer et al., 1994; Davoren et al., 1986; El-Roeiy et al., 1993; El-Roeiy et al., 1994), thecal cells of cattle, humans and rats (Hernandez et al., 1988a, Hernandez et al., 1988b, Bergh et al., 1993, El-Roeiy et al., 1993; El-Roeiy et al., 1994; Stewart et al., 1995), luteal cells of cattle and rats (Ladenheim et al., 1984, Sauerwein et al., 1992, Parmer et al., 1991; Samoto et al., 1993a; 1993b; Talavera and Menon, 1991), and ovarian stromal cells of human and rats (Jarrett et al., 1985; Poretsky et al., 1988; Hernandez et al., 1992).

Among the various actions of insulin in the ovary, the stimulatory effects seem to be predominant. In vitro, insulin has been reported to increase proliferation and(or) DNA synthesis in bovine, porcine and ovine granulosa cells (Spicer et al., 1993; Baranao and Hammond, 1984; Gong et al., 1993c; Monniaux and Pisselet, 1992). Chakravorty et al. (1993) reported that insulin was less potent than IGF-I in stimulating DNA synthesis in cultured bovine luteal cells and that their effects were not additive, suggesting a common mechanism of action. In vivo, PMSG-stimulated prepubertal gilts treated with insulin have increased numbers of small (but not medium or large) follicles (Matamoros et al., 1991). In cyclic heifers treated with GH, serum insulin concentrations were significantly

correlated with the number of small follicles (Gong et al., 1993a). In contrast, in superovulated cattle, insulin treatment had no effect on the numbers of antral follicles of any size category (Simpson et al., 1994).

Insulin has a stimulatory effect on estrogen production by granulosa cells, however there are differences among species. In rats and primates, insulin can stimulate estradiol production by granulosa cells in vitro, nonetheless its relative potency suggests that this effect is mediated via type I IGF receptors (Adashi et al., 1989; Giudice , 1992; Erickson et al., 1990). However, in cattle, insulin is a more potent stimulator of estradiol production by granulosa cells than IGF-I (Spicer et al., 1993a; Spicer et al., 1994 ; Gong et al., 1994). Also, in vivo treatment of superovulated cattle with insulin increased follicular fluid concentrations of estradiol in large follicles (Simpson et al., 1994). In contrast, estradiol production by porcine granulosa cells is not affected (Maruo et al., 1988) or is actually inhibited (Veldhuis et al., 1983) by insulin treatment in vitro.

Several studies indicate that insulin stimulates progesterone production by bovine granulosa cells (Spicer et al., 1993a; McArdle et al., 1991). In addition, it appears that insulin can stimulate progesterone production by bovine (Stewart et al., 1995) and porcine (Caubo et al., 1989; Morley et al., 1989) thecal cells, as well as progesterone production by luteal cells of cattle and rats (Mc Ardle et al., 1989, 83; Ladenheim et al., 1984; Sauerwein et al., 1992). Insulin has been shown to have stimulatory effects on LH-induced thecal cell androgen biosynthesis in pigs (Morley et al., 1989), rats (Hernandez et al., 1988a; Simone and Mahesh, 1993), humans (Bergh et al., 1993) and cattle (Stewart et al., 1995). Thus, it appears that insulin is stimulatory to granulosa and luteal cell progesterone production and thecal cell androstenedione production regardless of species.

REPRODUCTIVE FUNCTION OF BOS TAURUS AND BOS INDICUS CATTLE

Reproductive performance

Although Bos taurus and Bos indicus cattle probably descended from common ancestors in Asia (Sanders, 1980), several differences in the reproductive characteristics between these genotypes have been reported. Angus cows, bred in the spring under semitropical conditions, had greater pregnancy rates at first service and at 75 days postpartum than Brahman cows (Reynolds et al., 1979). Gestation length averaged 11 days shorter in Angus cows (Foote et al., 1960; Sagebiel et al., 1973) than in Brahman cows (Plasse et al., 1968b; Reynolds et al., 1980) and calf survival rate was less in Angus than in Brahman cows (Peacock et al., 1977; Turner et al., 1968; Reynolds et al., 1980). However, the twinning rate in Bos taurus cows is greater than in Bos indicus cows (Rutledge, 1975). Duration of estrus is shorter, less intense, and occurs later in ovariectomized Bos indicus females than in ovariectomized Bos taurus females given estradiol (Randel, 1984; Plasse et al., 1970; Galina et al., 1982; Rhodes and Randel, 1978; Rhodes et al., 1978). In contrast, Bos indicus heifers ovulated earlier after the onset of estrus than Bos taurus heifers (Randel, 1984), and the interval to the first postpartum estrus is longer in Bos indicus females than in Bos taurus females (Reynolds, 1979; Plasse et al., 1970). Moreover, the occurrence of estrous behavior in the spring in a semitropical environment was significantly greater in Angus than in Brahman cows (Reynolds et al., 1979). Furthermore, puberty is reached later in Bos indicus than in Bos taurus cattle (Plasse et al., 1968a).

Follicular growth and development

Although little research has been done comparing the patterns of follicular growth and development between Bos taurus and Bos indicus cattle, some differences have been reported. The number of small follicles (< 5 mm) is greater in Brahman cows than in Angus cows on day 17 of the cycle , whereas, the number of large follicles (> 5 mm) is greater in Angus than in Brahman (Segerson et al., 1984). In another study (Simpson et al., 1994), superovulated Brahman cows had more medium (4.0 to 7.9 mm) and approximately twice the number of total follicles than did superovulated Angus cows, however, there was no difference in the number of small follicles (1.0 to 3.9 mm) nor large follicles (≥ 8 mm). Differences in the response to superovulation treatment between breeds or in the time of ovariectomy between the two studies may account for these discrepancies.

Luteal development

Morphology of the CL differs between Bos taurus and Bos indicus cattle. Because the CL is smaller, more deeply imbedded in the ovarian stroma, and less distinctive in Brahman than in Hereford heifers, the CL is more difficult to be detected by rectal palpation (Irvin et al., 1978; Plasse et al., 1968a; Rhodes et al., 1978). Weight of the CL was greater on day 13 of the estrous cycle in Hereford heifers than in Brahman heifers (Rhodes et al., 1978; Irvin et al., 1978). Similarly, on day 17 of the estrous cycle , the CL was heavier in Angus than in Brahman cows (Segerson et al., 1984). Thus, CL size appears larger in Bos taurus versus Bos indicus cattle.

Endocrine profiles

Comparisons between the reproductive endocrine profiles of Bos taurus and Bos indicus cattle have revealed several differences. The patterns of LH secretion may differ between Bos indicus and Bos taurus. Specifically, Brahman cows (both intact and ovariectomized-estrogen-challenged) have less basal LH concentrations, lower preovulatory peaks of LH, and a shorter length of the preovulatory LH surge than Hereford cows (Rhodes et al., 1978). Bos indicus (Brahman) cows had less mean LH concentrations on day 40 and 50 postpartum than Bos taurus (Hereford x Shortorn) cows (D,Occhio et al., 1990). However, Griffin and Randel (1978) did not find any differences in overall mean serum LH concentrations between ovariectomized Brahman and Hereford cows, but concluded that ovariectomized Brahman cows were significantly less responsive to GnRH -induced LH release than ovariectomized Hereford cows. In postpartum cows, no differences in plasma FSH concentrations were found between Bos indicus (Brahman) and Bos taurus (Hereford x Shortorn) cows (D'Occhio et al., 1990).

Steroid secretion by the ovary is different between Bos taurus and Bos indicus cattle. Concentrations of progesterone in plasma during the luteal phase were greater in Brown Swiss (Bos taurus) and Holstein (Bos taurus) than in White Fulani (Bos indicus) cattle (Adeyemo and Heath ,1980). This is consistent with results of Segerson et al (1984) who found serum progesterone concentrations on day 7 to 17 were higher in Angus (Bos taurus) than in Brahman (Bos indicus) cows. However, (Adeyemo and Heath, 1980) did not find any differences in serum progesterone concentrations during the follicular phase of the estrous cycle of Bos taurus and Bos indicus heifers. Similarly,

(Rhodes et al., 1982) did not found differences during the luteal phase between Bos indicus and Bos taurus heifers. Serum estradiol concentrations from day 7 through day 17 were greater in Angus than in Brahman cows (Segerson et al., 1984), and in another study (Simpson et al., 1994), follicular fluid concentrations of estradiol in medium and large follicles of Angus cows were greater than those of Brahman cows. Thus, luteal progesterone secretion and follicular estradiol secretion seems to be greater in Bos taurus than in Bos indicus cattle.

Concentrations of metabolic hormones may differ among Bos taurus and Bos indicus genotypes. Plasma concentrations of IGF-I were over twofold greater in superovulated Brahman than in Angus cows (Simpson et al., 1994). Similarly, follicular fluid concentrations of IGF-I in medium and large follicles were greater in Brahman than in Angus cows after superovulation (Simpson et al., 1994). Also, plasma IGFBP activity and insulin concentrations were greater in Brahman than in Angus cows during superovulation (Simpson et al., 1994). Whether these metabolic hormone differences exist between Bos indicus and Bos taurus cattle during a normal estrous cycle is unknown.

CONCLUSIONS

In cattle, folliculogenesis occurs in waves. During the estrous cycle in cattle, groups of follicles undergo series of growth and atresia before a follicle ovulates. Folliculogenesis in cattle is a complex process regulated by a complex network of hormones and growth factors. Gonadotropins (FSH and LH) are necessary to stimulate

follicular growth and the production of steroid hormones in the ovary (estradiol and progesterone). The somatotropic axis (GH, IGF-I, IGFBPs and insulin) also plays a role in folliculogenesis by stimulating follicular growth and steroidogenesis. Bos taurus and Bos indicus cows differ in several reproductive characteristics, however comparisons of the pattern of folliculogenesis and the hormones and factors involved in those two species of cattle have not been reported.

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CHAPTER III

OVARIAN AND ENDOCRINE ACTIVITY IN ANGUS, BRAHMAN AND SENEPOL COWS IN A SUBTROPICAL ENVIRONMENT

Abstract

To determine breed differences in ovarian follicular activity and luteal development, daily rectal ultrasonography was conducted on multiparous lactating Angus (temperate Bos taurus; n=12), Brahman (tropical Bos indicus; n=12) and Senepol (tropical Bos taurus; n=12) cows during an estrous cycle. Blood was also collected to determine plasma concentrations of FSH, LH, progesterone, estradiol, GH, IGF-I, IGF-II, IGFBPs, insulin, glucose, and PUN. Number of small (2 to 5 mm) follicles was greater ($P < 0.05$) in Brahman (40 ± 1) than in Angus (21 ± 1) and Senepol (33 ± 1) cows and was greater ($P < 0.001$) in Senepol than in Angus cows. Number of medium (6 to 8 mm) follicles was less ($P < 0.001$) in Angus (2.3 ± 0.2) than in Brahman (5.0 ± 0.2) or Senepol (3.9 ± 0.2) cows. Number of large (≥ 9 mm) follicles was greater ($P < 0.001$) in Brahman (1.6 ± 0.1) than in Angus (0.9 ± 0.1) or Senepol (1.2 ± 0.1) cows and increased ($P < 0.05$) from day 1 (day of ovulation) to day 20 of the estrous cycle in all breeds. The maximum size of the first dominant and ovulatory follicles were greater ($P < 0.001$) in Brahman than in Angus

and Senepol cows. No differences ($P > 0.10$) in growth rate of the ovulatory follicle were found among breeds and were 1.39 ± 0.1 mm/day, 1.35 ± 0.1 mm/day, and 1.46 ± 0.1 mm/day for Angus , Brahman, and Senepol cows, respectively. The number of follicular waves per cycle tended to be different ($P = 0.145$) among genotypes; Angus and Brahman cows had predominantly two-wave cycles (72.7 % and 55.6 %, respectively) whereas Senepol cows had mostly (70.0 %) three-wave cycles. Length of the estrous cycle was greater ($P < 0.05$) in Senepol (21.8 ± 0.6 days) than in Angus (18.6 ± 0.6 days) or Brahman (19.5 ± 0.6 days) cows. Preovulatory surges of estradiol occurred earlier in Angus and Brahman cows than in Senepol (breed x day, $P < 0.07$) and were greater ($P < 0.005$) on day 18 to 20 than on day 2 to 16 (day effect, $P < 0.001$). Diameter of the CL imcreased ($P < 0.001$) from day 2 to 14 and was greater ($P < 0.001$) in Brahman (16.4 ± 0.3 mm) and Senepol (16.3 ± 0.3 mm) than in Angus (15.0 ± 0.3 mm) cows. Maximum CL diameter was greater ($P < 0.05$) in Senepol (22.2 ± 0.3 mm) than in Angus (19.6 ± 0.2 mm) cows and tended to be greater ($P = 0.10$) in Brahman (21.3 ± 0.3 mm) than in Angus cows. Mean progesterone concentrations increased ($P < 0.001$) from day 2 to 14 of the estrous cycle but did not differ ($P > 0.10$) among genotypes. There was a breed effect ($P < 0.01$), a day effect ($P < 0.001$) and a breed x day interaction ($P < 0.05$) for plasma concentrations of FSH; secondary surge of FSH between day 1 and 2 was more pronounced in Angus than Brahman or Senepol cows. Mean concentrations (day 2 to 14 of the estrous cycle) of LH were not different ($P > 0.10$) among Angus (4.28 ng/ml), Brahman (4.13 ng/ml) and Senepol (4.37 ng/ml) cows and did not change ($P > 0.10$) with day of estrous cycle. Concentrations of GH, IGF-I and insulin in plasma did not change

between days 0 and 20, and were greater ($P < 0.001$) in Brahman than in Angus or Senepol cows. Plasma concentrations of IGF-II did not differ ($P > 0.10$) among genotypes or between day 0 and day 10. Ligand blotting with ^{125}I-IGF-II revealed at least five forms of IGFBP: 40-44 kDa (IGFBP-3), 34 kDa (IGFBP-2), 27-29 kDa, 22 kDa, and 20 kDa. Binding activities of IGFBP-3, IGFBP-2, and the 27-29-kDa IGFBP did not differ ($P > 0.10$) among genotypes and averaged 13.1 ± 1.0, 7.4 ± 0.5, and 6.5 ± 0.5 arbitrary densitometric units, respectively. Brahman cows tended ($P < 0.10$) to have greater 22-kDa and 20-kDa IGFBP binding activities (3.5 ± 0.4 and 1.0 ± 0.2 units) than Angus (2.6 ± 0.4 and 0.5 ± 0.2 units) or Senepol (2.3 ± 0.4 and 0.3 ± 0.2 units) cows. Concentrations of glucose in plasma were greater ($P < 0.01$) in Senepol (77.0 ± 0.6 mg/dL) than in Brahman (74.5 ± 0.7 mg/dL) and Angus (68.9 ± 1.1 mg/dL) cows, and were also greater in Brahman ($P < 0.05$) than in Angus. There was a day effect ($P < 0.01$) for the plasma concentrations of glucose and PUN. Plasma urea nitrogen concentration was greater ($P < 0.001$) in Brahman (15.4 ± 0.2 mg/dL) cows than in Angus (11.8 ± 0.2 mg/dL) and Senepol (14.6 ± 0.2 mg/dL) cows. In conclusion, Brahman (Bos indicus) cows as well as Senepol cows (tropical Bos taurus) had greater number of follicles in all size categories and larger CL than Angus (temperate Bos taurus) cows. These differences may be due to differences in the pattern of secretion of endocrine and metabolic hormones.

Introduction

Differences in the reproductive characteristics between Bos taurus and Bos indicus cattle have been reported. Gestation length is shorter in Angus (Foote et al., 1960; Sagebiel et al ., 1973) than in Brahman cows (Plasse et al., 1968; Reynolds et al., 1980). Estrus in Bos indicus females is shorter and less intense than in Bos taurus females (Rhodes and Randel, 1978; Rhodes et al., 1978; Galina et al., 1982; Randel, 1984). In addition, puberty is reached later in Bos indicus than in Bos taurus cattle (Plasse et al., 1968; Baker et al., 1989), and twinning rate is much lower in Bos indicus than in Bos taurus cattle (Rutledge, 1975). Disparities in several reproductive parameters including gonadotropin and steroid secretion, follicular growth and luteal development, and/or the secretion of metabolic hormones may account for these differences in reproductive performance. Brahman cows have less basal LH concentrations, a lower preovulatory surge of LH and a shorter duration of the preovulatory surge than Hereford cows (Rhodes et al., 1978). Mean plasma progesterone (P4) concentrations during the luteal phase are greater in Bos taurus (Holstein and Brown Swiss) than in Bos indicus (White Fulani) cattle (Adeyemo and Heath, 1980; Segerson et al., 1984). Likewise, serum estradiol concentrations from day 7 to 17 are greater in Angus (Bos taurus) than in Brahman (Bos indicus) cows (Segerson et al., 1984). In addition, number of small follicles is greater in Brahman than in Angus cows on day 17 of the estrous cycle (Segerson et al., 1984), and superovulated Brahman cows had more medium and approximately twice the number of small follicles than superovulated Angus cows (Simpson et al., 1994). Moreover, plasma

concentrations of insulin-like growth factor-I (IGF-I) were over two-fold greater in Brahman than in Angus cows (Simpson et al., 1994).

Senepol cows (tropically adapted Bos taurus) were developed in the early 1900's in the Virgin Islands from a cross between African (N'Dama) and European (Red Poll) breeds of cattle (Williams et al., 1988). Several traits such as good temperament, calving ease, and heat resistance make Senepol cattle a suitable breed for beef production in subtropical environments (Hupp, 1981; Hammond et al., 1996). However, reproductive characteristics of Senepol cattle have not been reported. Ovarian function and endocrine secretion have not been compared among Angus, Brahman and Senepol cows. Therefore, the objectives of this study were to compare ovarian function (follicular growth and luteal development) and endocrine secretion among Angus, Brahman and Senepol cows maintained in a subtropical environment.

Materials and Methods

Animals, management and estrous synchronization

The study was carried out during the summer (July and August) of 1995 at the STARS in Brooksville, FL. Twelve multiparous lactating cows were randomly selected from each of the Angus (temperate Bos taurus; age = 6.5 ± 0.4 years; BW = 407 ± 9 kg; BCS = 5 ± 0.3), Brahman (tropical Bos indicus; age = 6.9 ± 0.4 years; BW = 490 ± 10 kg; BCS = 5.2 ± 0.3) and Senepol (tropical Bos taurus; age = 6.2 ± 0.4 years; BW = 478 ± 8 kg; BCS = 4.7 ± 0.3) cow herds at the STARS in Brooksville, Florida. Cows and calves

were housed in a drylot as one group. Rhizoma perennial peanut-grass hay and minerals were fed free choice. Estrus was synchronized using prostaglandin-$F_{2\alpha}$ (PGF; Lutalyse®, The Upjohn company, Kalamazoo, MI). The synchronization procedure consisted an injection of 25 mg PGF (i.m.), and 11 and 12 days later another injection of 12.5 mg PGF on each day. Cows were visually observed for signs of estrus for 1 h at 12-h intervals from 1 to 4 days after the last injection of PGF. Sterile bulls fitted with chinball markers were used to assist in the detection of estrus. Estrus begins when a cow stands to be mounted by another cow or marker bull. Observations for estrus resumed 17 days after estrus to determine the length of the estrous cycle. Five cows were removed from the original sample group; one cow was discarded due to the presence of a cystic follicle, two of the cows failed to exhibit estrus behavior, and two other cows had irregular cycle lengths and irregular P4 profiles.

Ovarian ultrasonography

Beginning on day 1 (day of ovulation) of the synchronized estrous cycle and continuing daily through a subsequent estrous cycle (i.e., two estrous cycles), ovaries were examined by ultrasonic imaging (Aloka 210 ultrasound scanner equipped with a 7.5 mHz probe, Corometrics Medical Systems, Inc., Wallingford, CT) through the wall of the rectum. Each ultrasonography was recorded on video tape using a VCR (Mitsubishi HS-U510, Mitsibishi Electronics America, Inc.Norcross, GA). Tapes were projected on a monitor and a diagram depicting the relative location of follicles ≥ 2 mm and the corpus luteum (CL) was drawn for each ovary. Numbers and sizes of ovarian follicles and size of the CL, height, width and cavity were determined at each session. Ovulation was

determined by the dissapearance of the dominant follicle and subsequent formation of a CL at the same location in the ovary. Maximum diameter and growth rate of the first dominant and ovulatory follicles were also estimated. Growth rate of the dominant follicle was determined from the day the dominant follicle was first identified to the day that the diameter of the follicle no longer increased more than 1 mm. Growth rate of the ovulatory follicle was determined from first detection to ovulation. Number of follicular waves for each cycle was determined using the identity method (Knopf et al., 1989) in which individual follicles on each ovary (>5 mm) are identified and assigned a position relative to the other ovarian structures present (i.e., CL and follicles). A wave of follicular growth was identified by the presence of a dominant follicle and a group of growing follicles (subordinates) associated with the dominant follicle. The day of emergence was defined as the last day a group of follicles were 4 mm as indicated by the increasing diameters on subsequent days. The dominant follicle was defined as the largest follicle present on either ovary before day 12, and a subordinate follicle was defined as one that originated from the same follicular pool as a dominant follicle and indicated by: (1) its first detection within 2 days of the first detection of the dominant follicle, and (2) a subsequent increase in diameter. The growing phase of a dominant and ovulatory follicle was defined as day of first detection to the first day of maximum growth, and for the ovulatory follicle, the period from detection to ovulation, respectively. Numbers of follicles were divided into three distinct size classes for analyses: small (2 to 5 mm), medium (6 to 9 mm) and large ($\geq$ 10 mm) follicles.

Blood collection and hormone analyses

On each day of ovarian ultrasonography during the second cycle after estrus synchronization (i.e., one cycle), a blood sample was collected from each cow by jugular venipuncture into 9-mL blood collection tubes containing EDTA (Monovette® Sarstedt, Newton, NC). Blood samples were placed on ice immediately after collection, and plasma was separated by centrifugation (4 °C, 300 x g for 5 min). Plasma samples were frozen and stored at (- 20° C) until metabolite and hormone analyses were performed. Plasma concentrations of glucose were quantified by an automated colorimetric method (Technicon AutoAnalyzer and II Industrial Method 339-19, Technicon Industrial Systems, Tarrytown, NY) based on the glucose oxidase procedure as described by Gochman and Schmitz (1972). Concentrations of plasma urea nitrogen (PUN) were measured by automated colorimetric procedure (Technicon AutoAnalyzer II Industrial Method 339-01, Technicon Industrial Systems, Tarrytown NY) based on the diacetyl monoxime method described by Marsh et al. (1965).

Concentrations of IGF-I in plasma were determined by a double-antibody radioimmunoassay (RIA) after acid-ethanol extraction as described previously (Echternkamp et al., 1990). Intra - and inter-assay coefficients of variation for five plasma IGF-I assays were 12.1% and 23.4 %, respectively. Sensitivity of the assay, defined as 90 % of total binding, was 0.98 ng/ml.

Plasma concentrations of IGF-II were determined by a double-antibody RIA after formic acid-acetone extraction as described previously (Spicer et al., 1995). All samples were run in one assay, and the intrassay coefficient of variation was 2.7% and sensitivity was 0.05 ng/tube.

Plasma concentrations of progesterone (P4) were determined using a solid-phase P4 RIA kit (Diagnostics Products Corporation, Los Angeles, CA) as previously described (Stewart et al., 1996). Intra- and inter-assay coefficients of variation for 11 assays were 4.4 % and 10.6 %, respectively. Sensitivity of the assay was 0.05 ng/ml.

FSH concentrations in plasma were quantified by a double-antibody RIA as previously described (Vizcarra et al., 1997). Intra- and inter-assay coefficients of variation for 10 assays were 11 % and 27.6%, respectively . Sensitivity of the assay was 0.013 ng/ml.

Insulin concentrations were measured using a solid-phase RIA kit (ICN Pharmaceuticals Inc., Costa Mesa, CA) as described previously (Simpson et al., 1994). Intra- and inter-assay coefficients of variation for 11 assays were 9.9 % and 13.5 %, respectively. Sensitivity of the assay was 0.112 ng/ml.

Concentrations of estradiol were determined using a solid-phase Serono Estradiol MAIA assay kit (Biodata SpA, Montecelio, Italy) after extraction with ethyl acetate as previously (Vizcarra et al., 1997). Intra- and inter-assay coefficients of variation for 9 assays were 15.0 % and 24.3 % respectively. Sensitivity of the assay was 0.625 pg/ml.

Growth hormone concentrations in plasma were quantified by a double-antibody RIA as previously described (Yelich et al., 1995). Intra- and inter-assay coefficients of variation for 5 assays were 8.0% and 15.8%. Sensitivity of the assay was 0.1 ng/ml.

Plasma concentrations of LH were measured by a double-antibody RIA as described by (Bishop and Wettemann, 1993). Intra- and inter-assay coefficients of variation for 5 assays were 12.1 % and 19.2%, respectively. Sensitivity of the assay was 0.1 ng/ml.

Ligand blots

Plasma IGFBP concentrations were analyzed by one dimensional SDS-PAGE, as previously described (Echternkamp et al., 1994). Briefly, samples were diluted 1:10 in PBS, of which 1.5 µl (added to 23.5 µl of buffer) was heat denatured and then separated on a 12% polyacrylamide gel via electrophoresis. After separation, proteins in gels were electrophoretically transferred to nitrocellulose, and ligand-blotted overnight with ^{125}I-IGF-II. After washing and then exposure to X-ray film at -70°C for 48 h, band intensity on autoradiographs was determined using a PDI Model DNA 35 scanner and Quantity One (version 2.4) software for quantification by scanning densitometry.

Statistical analyses

The experiment was a completely randomized design with repeated measures. The data were analyzed using PROC MIXED (SAS, 1996), with sources of variation including breed, cow within breed (error term for breed), day, breed by day interaction and residual. An autoregressive with lag equal to one model was used to model the covariance structure of the repeated measurements. If the breed by day interaction was significant, simple effects of breed were analyzed using the SLICE option for the LSMEANS statement. Satterthwait's approximation was used for calculation of the degrees of freedom of the pooled error term. If the breed by day interaction was not significant, the main effects were analyzed using LSMEANS with the DIFF option. For certain variables (Estradiol, CL diameter, P4, LH), the maximum value over the estrous cycle was found and that value represented the cow for that cycle. The resulting design was a completely

randomized design. PROC GLM was used to analyze these data with LSMEANS reported. Number of waves was analyzed by Chi-square procedure (SAS, 1996).

Results

Follicular dynamics and function

Number of small (2 to 5 mm) follicles was greater ($P < 0.05$) in Brahman (39 ± 2) than in Angus (21 ± 2) and Senepol (33 ± 2) cows and was greater ($P < 0.001$) in Senepol than in Angus cows (Fig 1); the day effect and breed x day interaction were not significant ($P > 0.10$). Number of medium (6 to 8 mm) follicles was less ($P < 0.05$) in Angus (2.3 ± 0.3) than in Brahman (5.0 ± 0.3) or Senepol (3.9 ± 0.3) cows (Fig. 2); the day effect and breed x day interaction were not significant ($P > 0.10$). Number of large (≥ 8 mm) follicles was greater ($P < 0.05$) in Brahman (1.6 ± 0.1) than in Angus (0.9 ± 0.1) or Senepol (1.2 ± 0.1) cows (Fig 3), but no breed x day interaction ($P > 0.10$) was found. There was a day effect ($P < 0.001$) on number of large follicles (Fig. 3). Number of large follicles in all breeds increased ($P < 0.05$) from day 1 (day of ovulation) to day 20 of the estrous cycle.

Maximum size of the first dominant follicle was greater ($P < 0.001$) in Brahman (15.3 ± 0.5 mm) than in Angus (12.8 ± 0.4 mm) cows, and was also greater ($P < 0.05$) in Senepol (13.9 ± 0.5 mm) than in Angus cows (Fig. 4). Growth rate of the first dominant follicle tended to be greater ($P < 0.10$) in Brahman (1.6 ± 0.1 mm/day) cows than in Angus (1.2 ± 0.1mm/day) and Senepol (1.2 ± 0.1 mm/day) cows (Fig. 5). Maximum size

of the ovulatory follicle was greater ($P < 0.001$) in Brahman (15.6 ± 0.4 mm) than in Angus (13.3 ± 0.4 mm) and Senepol (13.6 ± 0.4 mm) cows (Fig. 4). No differences ($P > 0.10$) in growth rate of the ovulatory follicle were found among breeds and were 1.39 ± 0.1 mm/day 1.35 ± 0.1 mm/day and 1.46 ± 0.1 mm/day, respectively, for Angus , Brahman and Senepol cows (Fig. 5). Chi-square analysis indicated a trend in the number of follicular waves during cycle 2 to be different ($P = 0.145$) among genotypes; Angus and Brahman cows had predominantly two-wave cycles (72.7 % and 55.6 %, respectively) whereas Senepol cows had mostly (70%) three-wave cycles (Table 1). Length of the estrous cycle was greater ($P < 0.05$) in Senepol (21.8 ± 0.6 days) than in Angus (18.6 ± 0.6 days) or Brahman (19.5 ± 0.6 days) cows. In both cycles (1 and 2), length of the estrous cycle was greater ($P < 0.005$) in 2-wave cows than in 3-wave cows.

Luteal development

Breed ($P < 0.001$), day ($P < 0.001$) and breed x day ($P < 0.06$) affected CL diameter across days 0 to 20. Regression of the CL occurred earlier in the estrous cycle of Angus and Brahman cows than in Senepol cows (Fig. 6). If data for CL diameter were analyzed for only days 2 to 14 of the estrous cycle, no significant ($P > 0.10$) breed x day interaction existed; CL diameter was greater ($P < 0.001$) in Brahman (16.4 ± 0.3 mm) and Senepol (16.4 ± 0.3 mm) than in Angus (15.0 ± 0.3 mm) cows. The diameter of the CL increased ($P < 0.001$) from day 1 to day 8 in all breeds (Fig. 6). Maximum diameter of the CL was greater ($P < 0.05$) in Senepol (22.4 ± 0.3 mm) than in Angus (19.6 ± 0.2 mm) cows and tended ($P = 0.10$) to be greater in Brahman (21.3 ± 0.3 mm) than in Angus

cows. No differences in maximum CL diameter were found between Brahman and Senepol cows.

Endocrine profiles

Day ($P < 0.001$) and breed x day ($P < 0.07$) but not breed ($P > 0.10$) affected plasma estradiol concentrations across days 0 to 20 of the estrous cycle. The preovulatory surge of estradiol occurred earlier in the estrous cycle of Angus and Brahman cows than Senepol cows. If data for plasma concentrations of estradiol were analyzed for only days 2 to 14 of the estrous cycle, the breed x day effect still existed ($P < 0.06$); this was due to a rise in plasma estradiol observed between days 2 and 5 in Angus and Brahman cows but not Senepol cows (Fig 7). Maximum plasma estradiol concentrations did not differ ($P > 0.10$) among Angus (8.6 pg/ml), Brahman (8.9 pg/ml) and Senepol 9.3 pg/ml) cows.

Day ($P < 0.001$), breed ($P < 0.05$) and breed x day ($P < 0.01$) affected plasma P4 concentrations across days 0 to 20. Luteal regression, indicated by a decrease in plasma P4, occurred earlier in Angus and Brahman cows than Senepol cows (Fig. 8). If data for plasma P4 concentrations were analyzed for only days 2 to 14 of the estrous cycle, no significant ($P > 0.10$) breed x day interaction existed and mean plasma P4 concentrations did not differ among breeds. Concentrations of P4 increased ($P < 0.001$) from day 0 to day 12 in all breeds(Fig. 8). Maximum plasma P4 concentration did not differ ($P > 0.10$) among Angus (4.3 ng/ml), Brahman (4.4 ng/ml) and Senepol (5.2 ng/ml) cows.

Breed ($P < 0.01$), day ($P < 0.001$) and breed x day ($P < 0.05$) affected plasma concentrations of FSH across days 0 to 20 of the estrous cycle. The secondary surge of FSH between days 1 and 2 was more pronounced ($P < 0.005$) in Angus (1.09 ± 0.08

ng/ml) than in Brahman (0.49 ± 0.08 ng/mL) and Senepol (0.58 ± 0.08 ng/ml) cows (Fig. 9).

Day ($P < 0.001$) and breed x day ($P < 0.01$) but not breed ($P > 0.10$) affected concentrations of LH in plasma across days 0 to 20 of the estrous cycle (Fig. 10). The preovulatory surge of LH occurred earlier in Brahman than Angus or Senepol cows (Fig. 10). If data for plasma concentrations of LH were analyzed for only days 2 to 14 of the estrous cycle, the breed x day and day effects were not significant ($P > 0.10$). Maximum plasma LH concentrations were not different ($P > 0.10$) among breeds and averaged 16.5, 30.0 and 21.2 ng/ml for Angus, Brahman and Senepol cows, respectively.

Concentrations of GH in plasma from day 0 to day 20 of the estrous cycle were greater ($P < 0.001$) in Brahman (42.2 ± 2.2 ng/ml) than in Angus (17.4 ± 2.1 ng/ml) or Senepol (19.7 ± 2.0 ng/ml) cows (Fig. 11). No significant day effect or breed x day interaction on plasma GH were observed ($P > 0.10$).

Plasma concentrations of IGF-I from day 0 to day 20 of the estrous cycle were greater ($P < 0.001$) in Brahman cows (22.9 ± 0.9 ng/ml) than in Senepol (14.6 ± 0.8 ng/ml) and Angus (10.0 ± 0.8 ng/ml) cows (Fig 12). Senepol cows had also greater ($P < 0.001$) concentrations of IGF-I than Angus cows. No significant day effect or breed x day interaction on plasma IGF-I was observed ($P > 0.10$). Plasma concentrations of IGF-II within each genotype, did not differ ($P > 0.10$) between day 0 (173 ± 7 ng/mL) and day 10 (169 ± 7 ng/mL). Plasma concentrations of IGF-II on day 0 and 10 did not differ ($P > 0.10$) among genotypes (Fig 13). No significant breed x day interaction on plasma IGF-II was observed ($P > 0.10$).

Ligand blotting with ^{125}I-IGF-II revealed at least five forms of IGFBP: 40-44 kDa (IGFBP-3), 34 kDa (IGFBP-2), 27-29 kDa, 22 kDa, and 20 kDa. Binding activities expressed in arbitrary densitometric units of IGFBP-3, IGFBP-2, and the 27-29-kDa IGFBP (Fig. 14) did not differ ($P > 0.10$) among genotypes and averaged 13.1 ± 1.0, 7.4 ± .5, and 6.5 ± .5, respectively. Brahman cows tended ($P < 0.10$) to have greater 22-kDa and 20-kDa IGFBP binding activity (3.5 ± 0.4 and 1.0 ± 0.2 units) than Angus (2.6 ± 0.4 and 0.5 ± 0.2 units) or Senepol (2.3 ± 0.4 and 0.3 ± 0.2 units) cows (Fig. 15).

Plasma concentrations of insulin from day 0 to day 20 of the estrous cycle were greater ($P < 0.001$) in Brahman (0.7 ± 0.05 ng/ml) than in Angus (0.4 ± 0.05 ng/ml) and Senepol (0.5 ± 0.05 ng/ml) cows, but no differences in plasma insulin concentrations existed between Angus and Senepol cows (Fig. 16). No significant day effect or breed x day interaction on plasma insulin were observed ($P > 0.10$).

Plasma metabolites

Concentrations of glucose from day 0 to day 20 of the estrous cycle in plasma were greater ($P < 0.05$) in Senepol (76.7 ± 0.6 mg/dL) than in Brahman (74.5 ± 0.7 mg/dL) and Angus (68.8 ± 1.1 mg/dL) cows (Fig. 17). There was a day effect ($P < 0.01$), but not a breed x day interaction on plasma concentrations of glucose indicating the decrease in glucose concentrations between days 0 and 8 of the estrous cycle was similar among breeds (Fig. 17).

Plasma urea nitrogen concentrations from day 0 to day 20 of the estrous cycle were greater ($P < 0.001$) in Brahman cows (15.4 ± 0.2 mg/dL) than in Angus (11.8 ± 0.2 mg/dL) and Senepol (14.6 ± 0.2 mg/dL) cows , and was also greater ($P < 0.001$) in

Senepol than in Angus cows (Fig. 18). There was a day effect ($P < 0.001$) but not a breed x day interaction ($P > 0.10$) on PUN concentrations indicating that the decrease in PUN concentrations between day 0 and 20 of the estrous cycle was similar among breeds (Fig. 18).

Discussion

Differences in reproductive characteristics exist among Angus, Brahman and Senepol cows. Follicular growth is greater in Bos indicus cattle than in Bos taurus as suggested by the greater number of follicles in all categories, maximum diameter of the first dominant and ovulatory follicles in Brahman than in Angus cows of the present study; in Senepol cows, follicular growth was intermediate between Angus and Brahman cows. The differences in follicle numbers are consistent with Segerson et al. (1984) who found a greater number of small follicles (< 5 mm) on day 17 of the estrous cycle in Brahman than in Angus cows and with Simpson et al. (1994) who found more medium (4.0 to 7.9 mm) follicles and approximately twice the number of total follicles in superovulated Brahman than Angus cows. The greater follicular activity in Brahman cows observed in the present study also included a greater maximum size of the ovulatory follicle in Brahman than Angus cows.

To our knowledge this is the first study in which the wave pattern of follicular growth in Senepol cows was determined. Senepol cows had mostly three-wave cycles, whereas the majority of Angus and Brahman cows had two-wave cycles. The percentage

of Brahman cows with two follicular waves (61 %) is greater in the present study than the 38 % reported by Zeitoun et al. (1996), but less than the 84 % reported by Figueiredo et al. (1997). Reasons for these discrepancies are unclear but may be due to the fact that our study was conducted during summer (June and July) in Florida, whereas Zeitoun et al. (1996) conducted their study during months of spring (May) and Fall (October) in Texas, and Figueiredo et al. (1997) conducted their study during winter months (July and August) in Brazil. Differences in nutritional status of the cows between studies could also account for the discrepancies. In our study, cows were fed peanut hay free choice whereas Zeitoun et al. (1996) and Figueiredo et al. (1997) supplemented their cows with concentrate. It has been reported that cows fed to loose body weight have predominantly 2-wave cycles whereas cows fed to gain body weight have 3-wave cycles (Murphy et al., 1991). Recently, seasonal differences in the number and size of antral follicles of Brahman cows have been reported (Lammoglia et al., 1996). The percentage of Senepol cows with two waves was less than in Angus cows. The longer interovulatory interval of the Senepol cows may account for the predominance of three-wave cycles in Senepol cows, because it has been suggested that the number of waves during the estrous cycle in cattle is regulated by the length of the luteal phase (Ginther et al., 1989). Generally, cycles with three or four waves have longer luteal phases than cycles with two waves (Sirois and Fortune, 1988; Ginther et al., 1989; Zeitoun et al., 1996), and artificially extending the luteal phase with exogenous progesterone has resulted in cycles with four or five waves of follicular growth (Sirois and Fortune, 1990).

In spite of the dramatic differences in follicle numbers and growth patterns that existed among genotypes, no differences in maximum plasma estradiol concentrations

were observed. However, the significant breed x day interaction for plasma estradiol concentrations revealed that an increase in estradiol concentrations occurred on day 5 in the estrous cycle of Angus and Brahman cows but not Senepol cows; this was likely due to the development of the first dominant follicle. The preovulatory rise in estradiol concentrations occurred earlier in Angus and Brahman cows than Senepol cows; peak (maximum) estradiol concentrations did not differ among breeds. In contrast, Segerson et al. (1984) found that estradiol concentrations from day 7 to 17 of the estrous cycle were less in Brahman than in Angus cows. Reasons for this discrepancy are unclear but may be due to: 1) the differences in the season in which samples were collected in our study (summer) versus the Segerson et al. (1984) study (Fall), 2) the fact that Angus heifers are less tolerant to heat than Brahman and Senepol heifers (Hammond et al., 1996), and(or) 3) the fact that Segerson et al., (1984) included pregnant and nonpregnant cows in their analysis. A greater concentration of estradiol in plasma of Brahman cows than in Angus cows would be expected due to the greater number of follicles in the former, however, Simpson et al. (1994) reported that follicles from superovulated Brahman cows contain less estradiol than those of Angus cows; this could explain why maximum plasma estradiol concentrations did not differ between Brahman and Angus cows in the present study. Similar plasma estradiol concentrations between Brahman and Angus cows in spite of a greater number of follicles in Brahman cows may be due to the fact that IGF-I , which was two-fold greater in plasma of Brahman than Angus cows, can inhibit insulin-stimulated estradiol production by granulosa cells of small and large bovine follicles (Spicer et al., 1994). Thus, Brahman cows with greater IGF-I concentrations may have reduced

estradiol production from follicles and needed more follicles to yield the same plasma concentration of estradiol as Angus cows.

In addition to greater concentrations of IGF-I, the greater follicular activity in Brahman cows could be explained by the greater concentrations of GH and(or) insulin than in Angus cows. The greater secretion of GH and IGF-I in Brahman than in Angus cows agrees with previous studies (Simpson et al., 1994; 1997). IGF-I stimulates granulosa cell proliferation and DNA synthesis in vitro (Baranao and Hammond, 1984; Monniaux and Pisselet, 1992; Spicer et al., 1993). Moreover, in heifers and lactating dairy cows treated with bovine GH, an increase in the number of small follicles was correlated with an increase in serum levels of both GH and IGF-I (Gong et al., 1992; 1993; Lucy et al., 1992). Also, in lactating dairy cows treated with GH, the number of large follicles (10 to 15 mm) on day 17 of the estrous cycle was greater than in non-treated cows, and the increase in number of follicles was correlated with an increase in follicular fluid IGF-I (Lucy et al., 1995). The number of medium (6 to 9 mm) and large follicles was greater in GH-treated lactating dairy cows (De La Sota et al., 1993; Lucy et al., 1995). However, in spite of the increased numbers of follicles after GH treatment, others (Schemm et al., 1990; Gong et al., 1991) did not find any differences between control and GH-treated cows in systemic estradiol concentrations which is consistent with findings of the present study. Treatment with GH, increases peripheral concentrations of IGF-I, insulin and GH significantly (Gong et al., 1991; 1993; Spicer et al., 1992; De La Sota et al., 1993; Stanko et al., 1994; Lucy et al., 1995), and thus sorting out the hormonal cause for increased follicular growth and steroidogenesis after GH treatment can be ambiguous. Insulin has also been proposed as a stimulator of folliculogenesis, increasing granulosa cell

proliferation and DNA synthesis in several species (Baranao and Hammond, 1984; Monniaux and Pisselet, 1992; Gong et al., 1993; Spicer et al., 1993). In vivo, treatment of prepubertal gilts with insulin increases the number of small but not medium or large follicles (Matamoros et al., 1991). Insulin increases the ovulation rate in gilts (Cox et al., 1987). However, exogenous insulin had no effect on the number of antral follicles in superovulated Angus and Brahman cows (Simpson et al., 1994). Thus, species differences may exist in terms of the in vivo effect of insulin on folliculogenesis.

To our knowledge this is the first time CL diameter in Bos taurus and Bos indicus cows have been compared using ultrasonography. We found that luteal growth was greater in Brahman and Senepol than in Angus cows as suggested by the larger size of the CL in Brahman and Senepol than in Angus cows. However, a smaller CL may be present in Bos indicus than in Bos taurus heifers (Irvin et al., 1978; Rhodes et al., 1982; Segerson et al., 1984). These discrepancies between the present and previous studies may be due to differences in timing and frequency of luteal measurements during the estrous cycle, and (or) differences in season in which the studies were conducted.

In spite of the greater CL diameter, no differences were found in P4 concentrations among breeds before day 14 of the estrous cycle. This agrees with previous studies that found no differences in plasma P4 concentrations (Rhodes et al., 1982) but contrasts with others that found less P4 concentrations (Adeyemo and Heath, 1980; Randel et al., 1984; Segerson et al., 1984) between Bos indicus and Bos taurus cattle. Differences in the sampling frequency, season and climate at time of sampling, and age and physiological state (e.g. lactating or non-lactating) of the animals may account for these discrepancies. Heat stress affects P4 production, because in Holstein cows, serum P4 between day 6 and

18 of the estrous cycle were less during summer than during spring (Howell et al., 1994). In addition, luteal P4 content was greater in Holstein cows exposed to forced ventilation than in cows not exposed (Younas et al., 1993). In agreement with previous studies (Kastelic et al., 1990; Rajamahendran and Taylor 1990; Ribadu et al., 1994) diameter and P4 concentrations were positively correlated in Angus ($r = 0.70$), Brahman ($r = 0.73$) and Senepol ($r = 0.75$) between day 0 and 14 of the estrous cycle of the present study. The length of the estrous cycle was greater in Senepol than Angus or Brahman cows and was associated with a prolonged luteal phase in Senepol cows.

The greater luteal growth in Brahman and Senepol cows versus Angus could be explained by the greater concentrations of GH and(or) IGF-I. Injections of GH in cattle that increased serum IGF-I concentration also increased size of CL and concentrations of P4 in plasma (Schemm et al., 1990; Gallo and Block, 1991; Lucy et al., 1994; Lucy et al., 1995). Luteal tissue in Bos indicus (Brahman) and Bos taurus (Hereford) cows have similar histological and morphological characteristics such as organization, apparent population of cells per area or cell types present (Irvin et al., 1978). However, in vitro, luteal tissue from Brahman (Bos indicus) cows secretes less P4 than luteal tissue from Hereford (Bos taurus) (Rhodes et al., 1982). The latter observation could explain why in our study, Brahman cows had bigger CL than Angus but yet plasma P4 concentrations were not different among those breeds; perhaps Brahman cows needed bigger CL to produce the same amount of P4. In addition to direct stimulatory effects of IGF-I on CL growth and steroidogenesis, direct effect of GH on the CL seems likely, since CL contained more GH receptor mRNA and GH receptor protein than other reproductive tissues (Lucy et al., 1993a ; Kirby et al., 1996).

Ligand blotting with ^{125}I-IGF-II revealed at least five forms of IGFBP 40-44 Kda (IGFBP3), 34 KDa (IGFBP-2), 27-29 kDa, 22 KDa, and 20 kDa. Genotype differences in IGFBP-3 and -2 activity were not found in the present study and this agrees with the study of Simpson et al. (1997) in which no differences were found in total IGFBP binding activity between ovariectomized Angus and Brahman cows but conflicts with Simpson et al. (1994) who found 10 % greater total plasma IGFBP activity in superovulated Angus than in Brahman cows. Differences in the season and frequency of sampling may account for the discrepancies between studies. Growth hormone has been implicated in the regulation of IGFBP-2 and -3 (Stanko et al., 1994). However, despite of the differences in circulating GH, no significant differences in IGFBP-2 were found among genotypes in the present study.

Differences in the hypothalamic-hypophyseal axis exist between Bos indicus and Bos taurus cattle (Rhodes et al., 1979). Ovariectomized Brahman cows produced less LH in response to GnRH than Hereford cows (Griffin and Randel, 1978). Also, Brahman heifers have significantly less LH during the preovulatory surge of LH, the LH surge occurs earlier, and they have a shorter interval from estrus to ovulation than Hereford cows (Randel, 1984). We found no differences in mean LH concentrations measured between days 2 and 14 of the estrous cycle or in maximum LH concentrations among Brahman, Angus and Senepol cows, but daily samples taken in the present study probably were not frequent enough to detect differences in the timing and magnitude of the LH surge. However, in agreement with the present study, Griffin and Randel (1978) did not find differences in mean serum LH levels, number and magnitude of LH peaks or maximum LH peak in ovariectomized Brahman and Hereford cows. Also, Rhodes et al.

(1978) did not find any differences in basal LH concentrations between ovariectomized Brahman and Hereford cows. Thus, differences in luteal function among breeds of the present study are not likely due to differences in luteal phase LH secretion but further research will be required to verify this suggestion.

The pattern of FSH secretion appears to differ among the three genotypes in the present study. Besides the greater mean plasma concentrations of FSH found in Angus cows of the present study, the secondary (postovulatory) peak of FSH was more distinct in Angus than Brahman or Senepol cows. The latter observation likely explains why the breed by day interaction in the mean concentration of FSH was significant. The greater concentrations of FSH in Angus cows may be due to less ovarian production of inhibin in Angus than in Brahman and Senepol cows. In prolific breeds of sheep, concentrations of inhibin produced by the ovary were lower and caused greater preovulatory surges of FSH than less prolific breeds (Cahill et al., 1981; Cummins et al., 1983; McNatty et al., 1987). Whether ovaries from Angus cows produced less inhibin than ovaries from Brahman and Senepol cows requires further evaluation. It has been reported that peaks of FSH occur before the rise of a follicular wave, and that the number of peaks is related to the number of waves in heifers (Adams et al., 1992), ewes (Ginther et al., 1995) and mares (Bergfelt and Ginther, 1993). Despite the greater plasma concentrations of FSH shown in Angus cows, the number of follicles was lower in Angus than Brahman or Senepol cows, and we suggest that this may be due to the lower IGF-I concentrations observed in Angus cows. We hypothesize that in the face of elevated FSH but low IGF-I, dominant follicles are more persistant and thus, fewer follicular waves develop during an estrous cycle. In the face of elevated IGF-I but low FSH, dominant follicles are less persistant and thus, more

follicular waves develop during an estrous cycle. However, a direct effect of FSH on follicular growth cannot be ruled out because FSH alone inhibits [^{3}H]thymidine incorporation into granulosa cells in small, medium and large follicles in a dose dependent manner; whereas IGF-I acts synergistically with FSH and LH to stimulate [^{3}H]thymidine incorporation into granulosa cells from small follicles (Gong et al., 1993). Another study reported that FSH alone had no effect on proliferation of granulosa cells from small follicles (Langhout et al., 1991).

Concentrations of glucose were greater in Brahman than in Angus cows. In contrast, Simpson et al. (1994; 1997) did not find any differences in plasma glucose concentrations in superovulated and estrogen-treated ovariectomized Brahman and Angus cows. Reasons for this discrepancy are unclear but may be due to differences in the type of ration fed between studies. The greater concentrations of insulin in plasma observed in our study also may be due to a different sensitivity to insulin among genotypes and it is further emphasized by the finding that Senepol cows had greater plasma glucose than Angus, yet no differences in plasma insulin concentrations between Senepol and Angus cows were observed.

The greater concentrations of PUN concomitant with greater concentrations of IGF-I observed in Brahman and Senepol versus Angus cows in this study is consistent with Simpson et al. (1994; 1997) who found greater PUN concentrations in Brahman than in Angus cows despite the greater plasma concentrations of IGF-I and GH in Brahman than Angus cows. Increased levels of PUN have been reported to be associated with decreased nitrogen retention and protein accretion in growing cattle (Enright et al., 1990; Hayden et al., 1993). In estradiol-implanted growing steers, treatment with GH increased

plasma concentrations of IGF-I and decreased PUN levels (Preston et al., 1995). Why greater plasma IGF-I concentrations are associated with increased PUN levels in mature cows whereas, in growing cattle, plasma IGF-I and PUN concentrations are negatively correlated will require further elucidation. Because Brahman and Brahman -crossbred cows produce more milk than Angus and Angus-crossbred cows (Brown et al., 1996; Vann et al., 1995; Green et al., 1991), perhaps Brahman cows were in a greater catabolic state than were Angus cows and thus had elevated PUN in the present study.

In conclusion, differences in follicular and luteal growth have been found between Brahman (Bos indicus), Senepol (tropically adapted Bos taurus) and Angus (temperately adpated Bos taurus). Differences in the endocrine patterns of secretion and response of the gonadotropic and somatotropic axis are responsible for those differences. Further research will be needed to establish the relative importance of these two systems on the ovarian activity of these breeds.

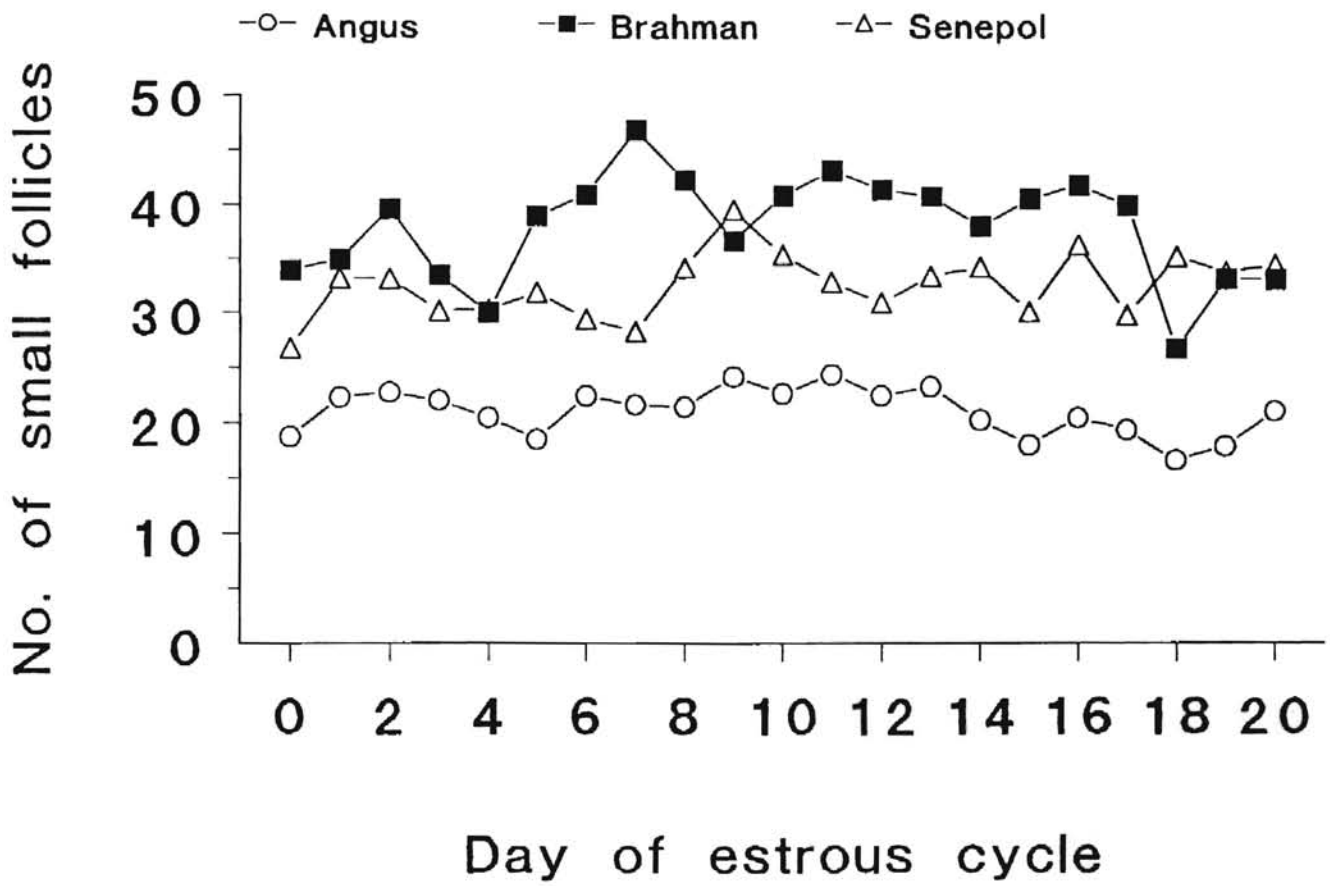

Fig. 1. Number of small follicles (2 to 5 mm) in Angus, Brahman, and Senepol cows during an estrous cycle as determined by daily rectal ultrasonography. Standard errors averaged over the estrous cycle were 3.7, 4.5 and 3.6 for Angus, Brahman and Senepol cows, respectively.

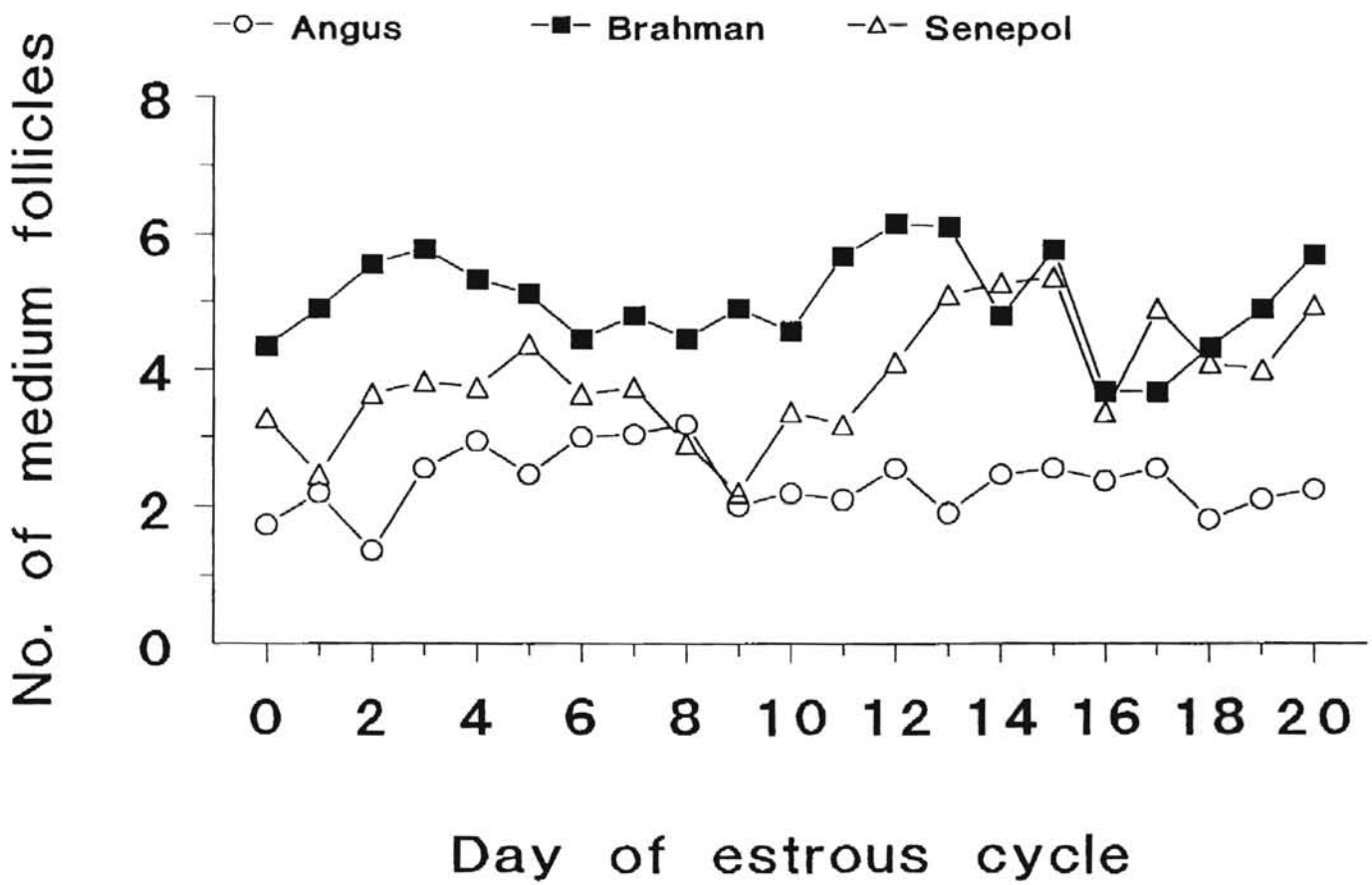

Fig. 2. Number of medium follicles (6 to 8 mm) in Angus, Brahman and Senepol cows during an estrous cycle as determined by daily rectal ultrasonography. Standard errors averaged over the estrous cycle were 0.7, 0.8 and 0.7 for Angus, Brahman and Senepol cows, respectively.

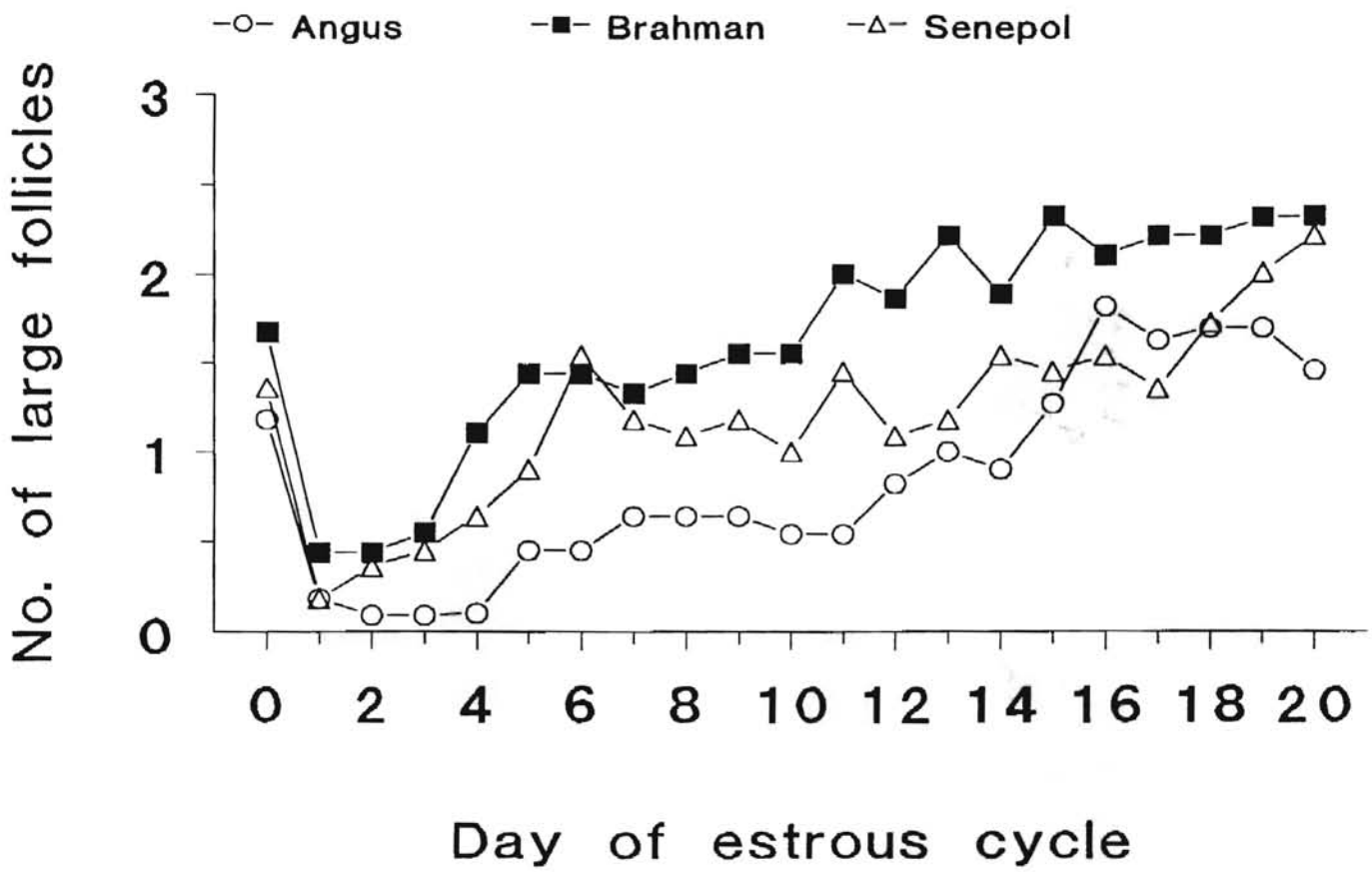

Fig 3. Number of large follicles (≥ 9 mm) in Angus, Brahman and Senepol cows during an estrous cycle as determined by daily rectal ultrasonography. Standard errors averaged over the estrous cycle were 0.2, 0.3 and 0.2 for Angus, Brahman and Senepol cows, respectively.

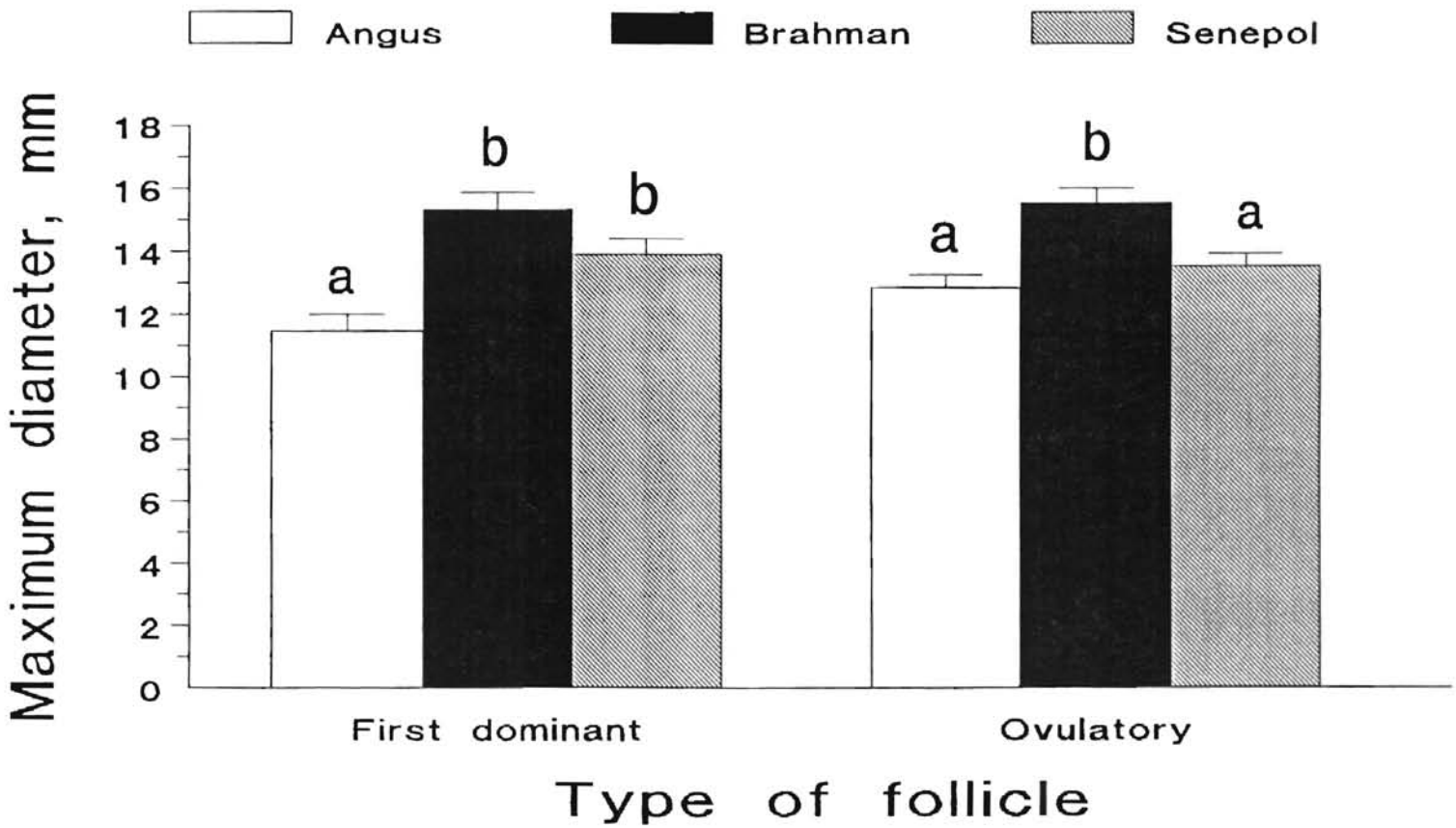

Fig. 4. Maximum diameter of the first dominant and ovulatory follicles in Angus, Brahman and Senepol cows during an estrous cycle as determined by rectal ultrasonography. [a, b] Within type of follicle means with different superscripts differ ($P < 0.05$).

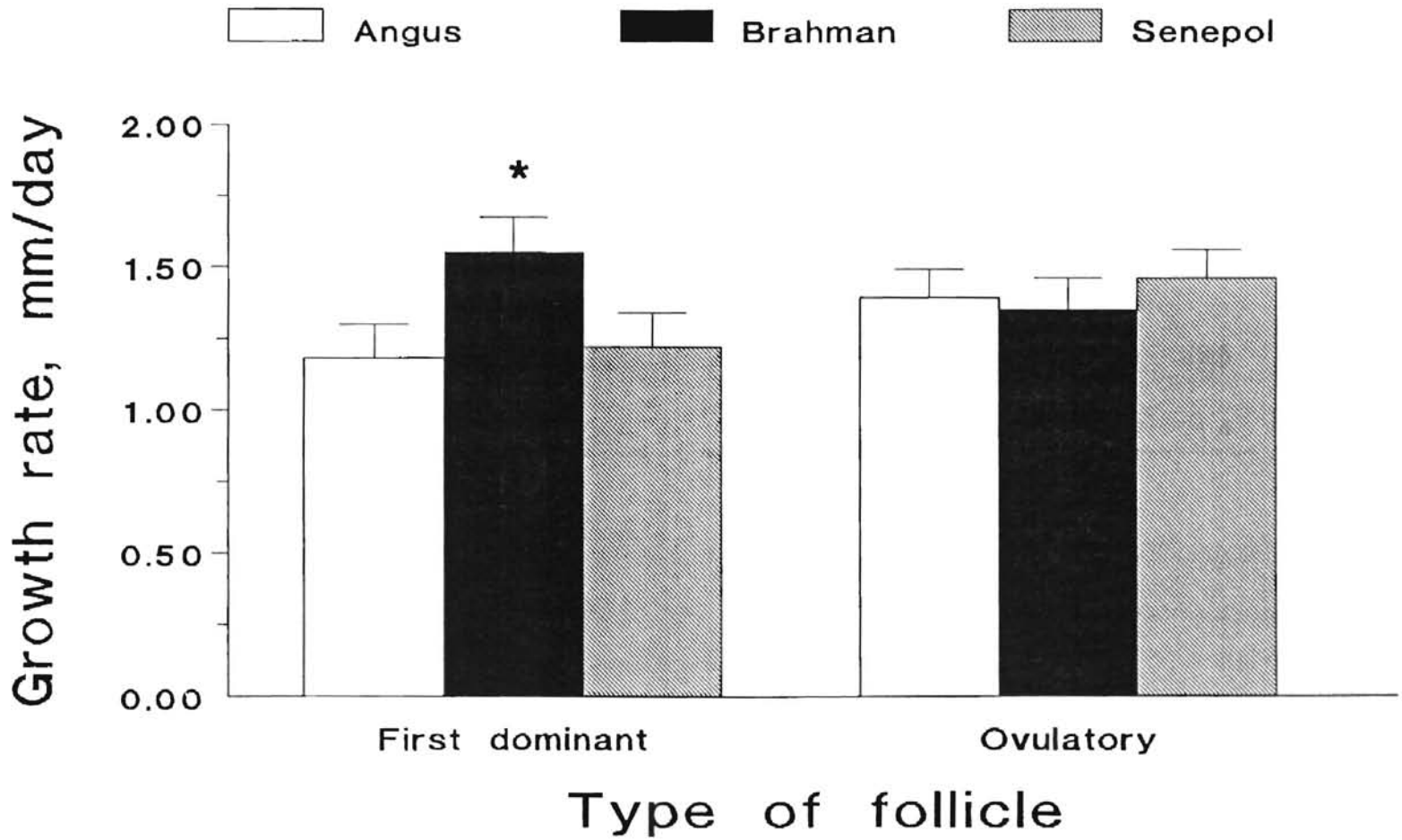

Fig. 5. Growth rate of the first dominant and ovulatory follicles in Angus, Brahman and Senepol cows during an estrous cycle as determined by rectal ultrasonography. *, $P < 0.10$ versus Angus and Senepol first dominant follicles.

Table 1. Number of follicular waves in Angus, Brahman and Senepol cows as determined by rectal ultrasonography

	Cycle 1					Cycle 2				
Breed	Number of cows	2 waves n (%)	Cycle length (days)	3 waves n (%)	Cycle length (days)	Number of cows	2 waves n (%)	Cycle length (days)	3 waves n (%)	Cycle length (days)
Angus	11	7 (63.6)	19.6	4 (36.4)	23.3	11	8 (72.7)	18.3	3 (27.3)	20.7
Brahman	9	6 (66.7)	20.0	3 (33.3)	21.3	9	5 (55.6)	18.6	4 (44.4)	20.8
Senepol	11	4 (36.4)	20.0	7 (63.6)	23.3	10	3 (30.0)	19.3	7 (70.0)	21.4

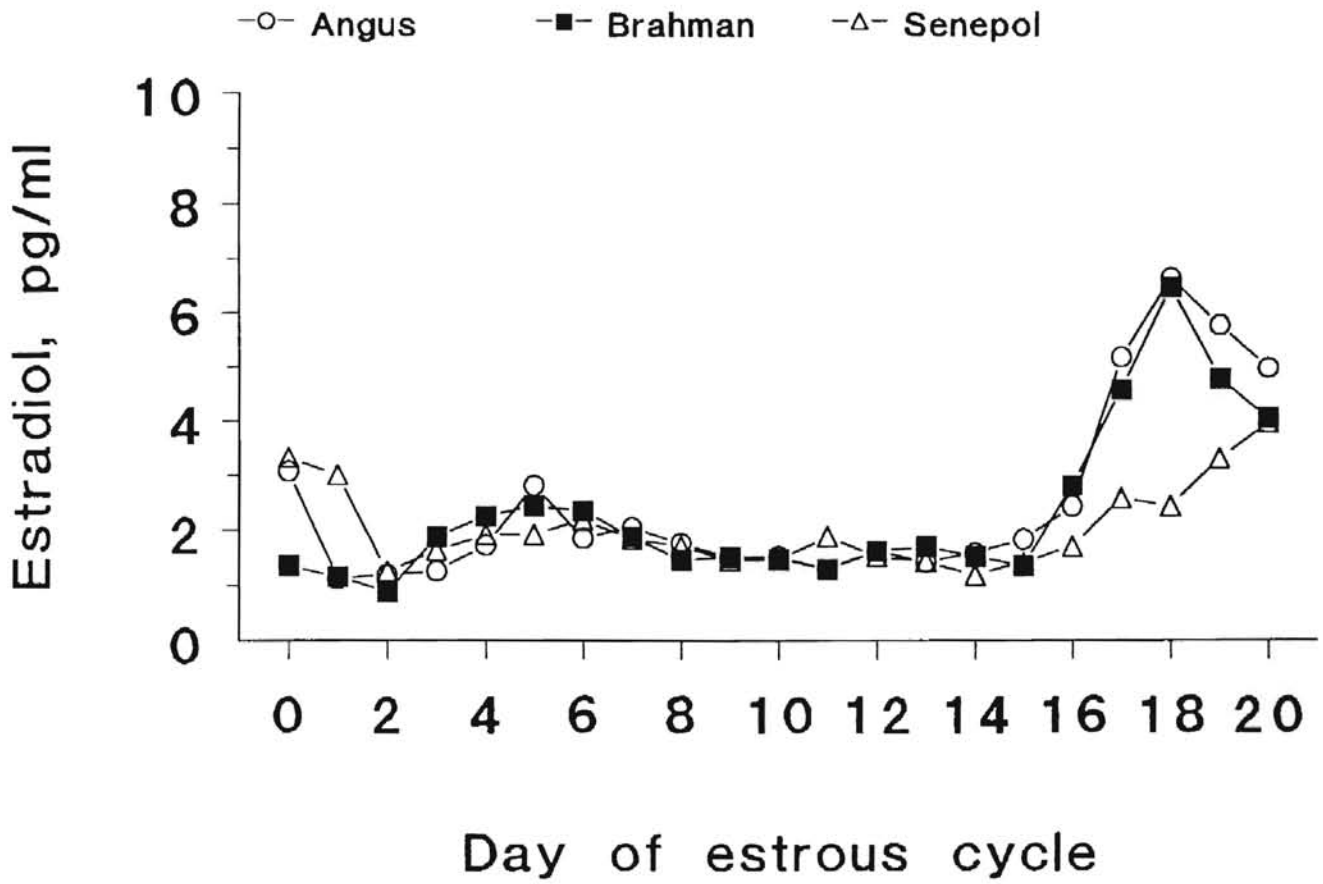

FIG 7. Plasma concentrations of estradiol in Angus, Brahman and Senepol cows as determined by RIA. Blood samples were collected daily. Standard errors averaged over the estrous cycle were 0.4, 0.4 and 0.3 for Angus, Brahman and Senepol cows, respectively.

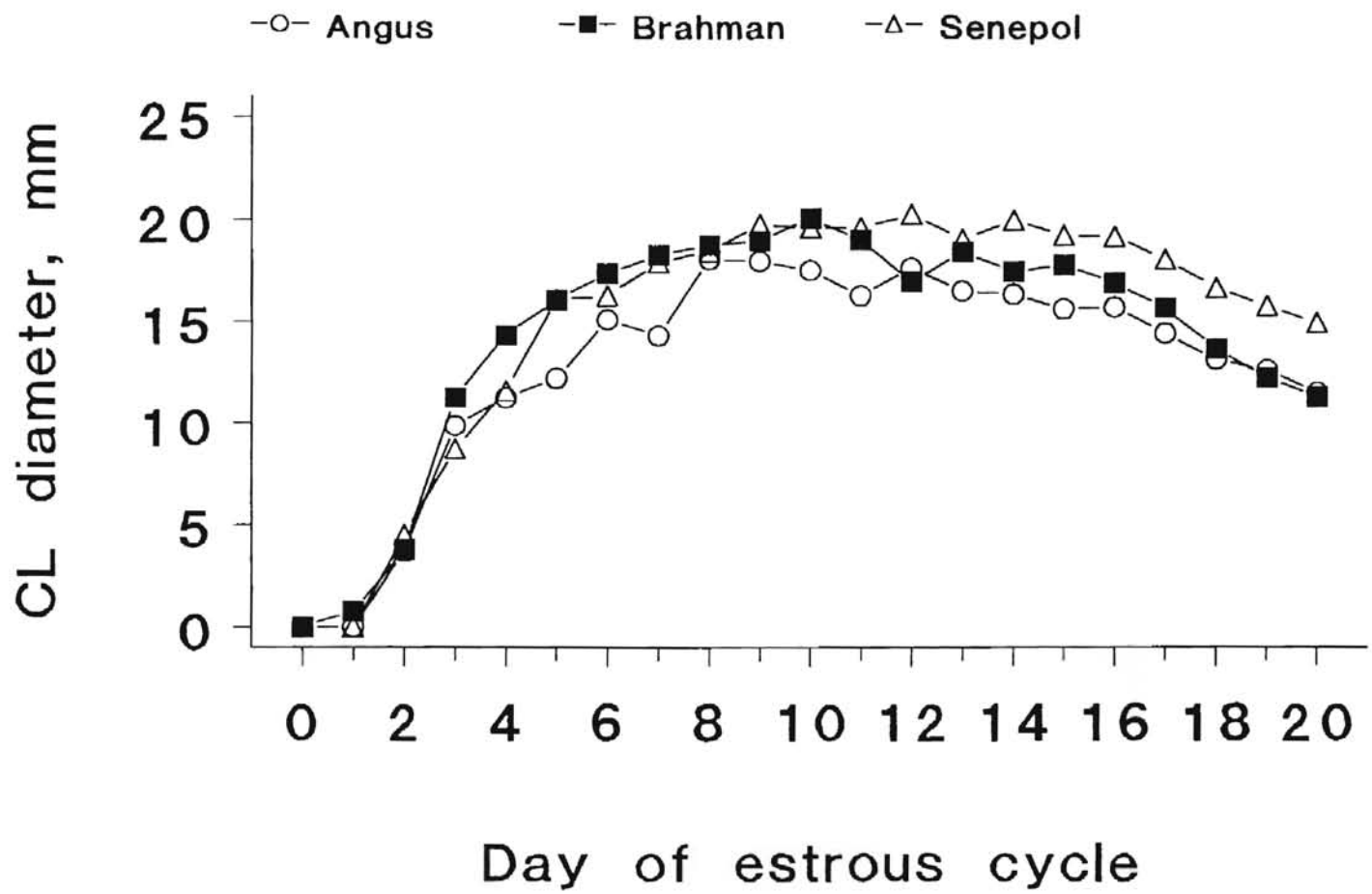

Fig. 6. Diameter of the CL in Angus, Brahman and Senepol cows during an estrous cycle as determined by daily rectal ultrasonography. Standard errors averaged over the estrous cycle were 0.9, 1.0 and 0.9 for Angus, Brahman and Senepol cows, respectively.

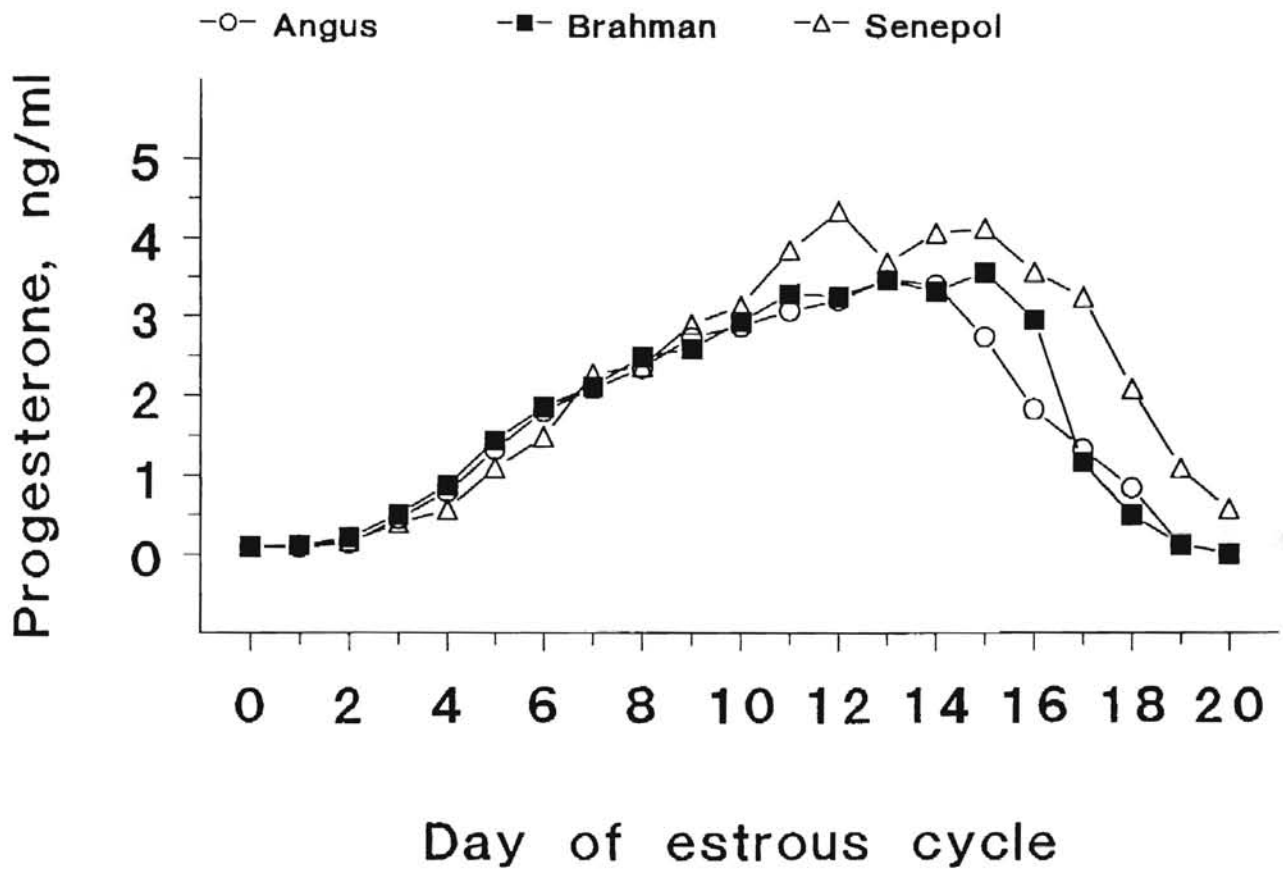

Fig. 8. Progesterone concentrations in Angus, Brahman and Senepol cows as determined by RIA. Blood samples were collected daily. Standard errors averaged over the estrous cycle were 0.2, 0.3 and 0.2 for Angus, Brahman and Senepol cows, respectively.

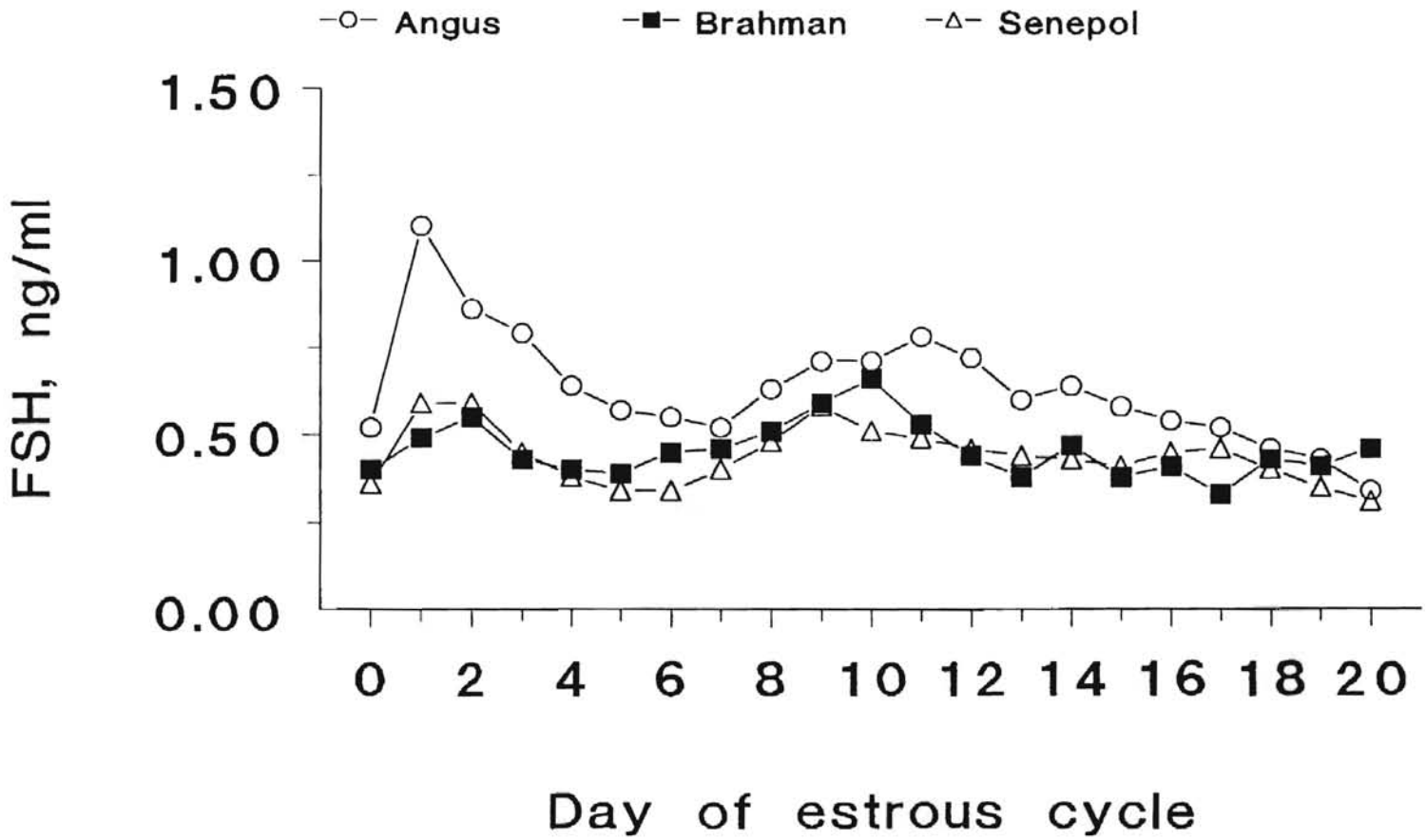

Fig. 9. Plasma concentrations of FSH in Angus, Brahman and Senepol cows as determined by RIA. Blood samples were collected daily. Standard errors averaged over the estrous cycle were 0.2, 0.1 and 0.2 for Angus, Brahman and Senepol cows, respectively.

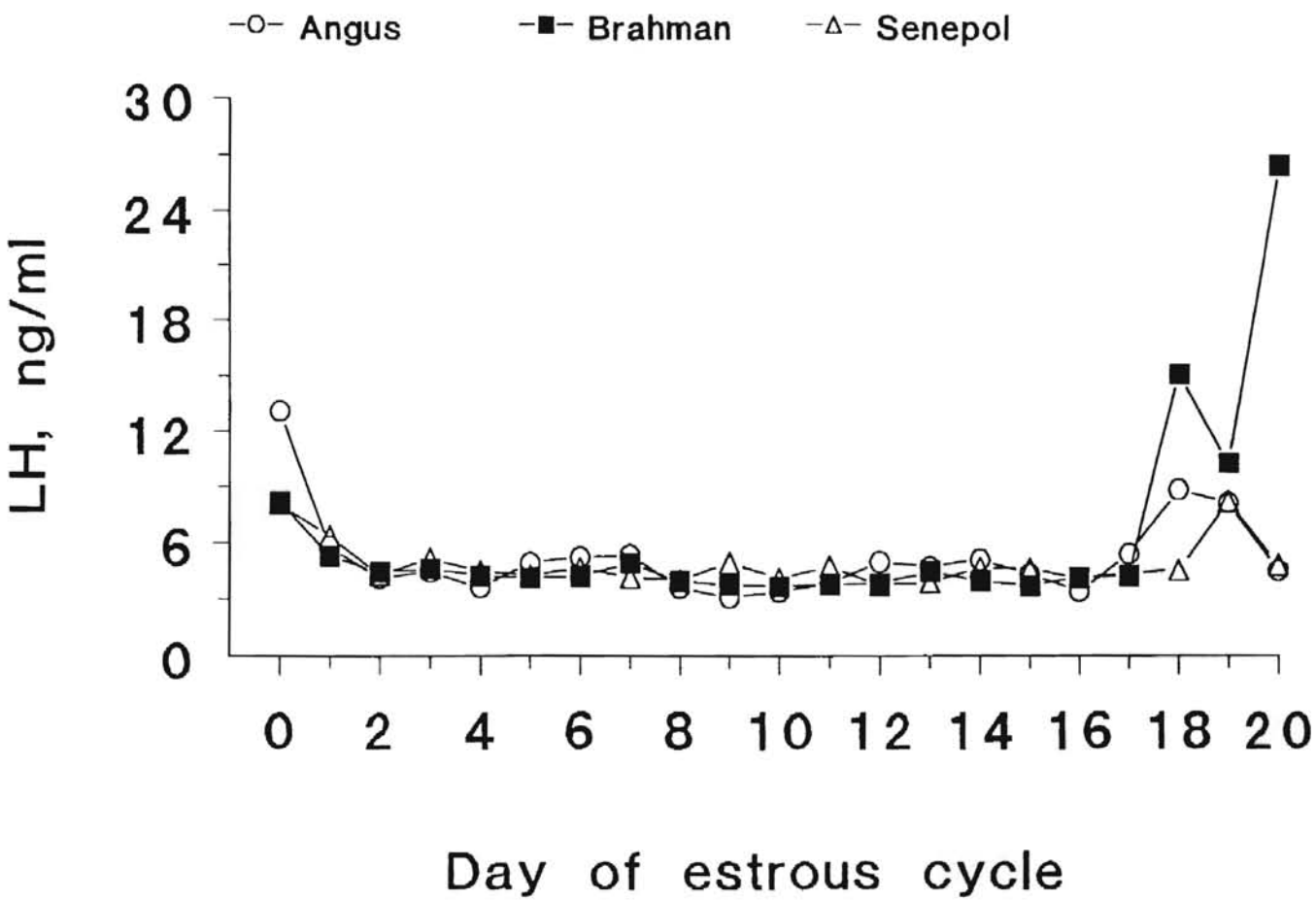

Fig. 10. Plasma concentrations of LH in Angus, Brahman and Senepol cows as determined by RIA. Blood samples were collected daily. Standard errors averaged over the estrous cycle were 0.5, 0.6 and 0.5 for Angus, Brahman and Senepol cows, respectively.

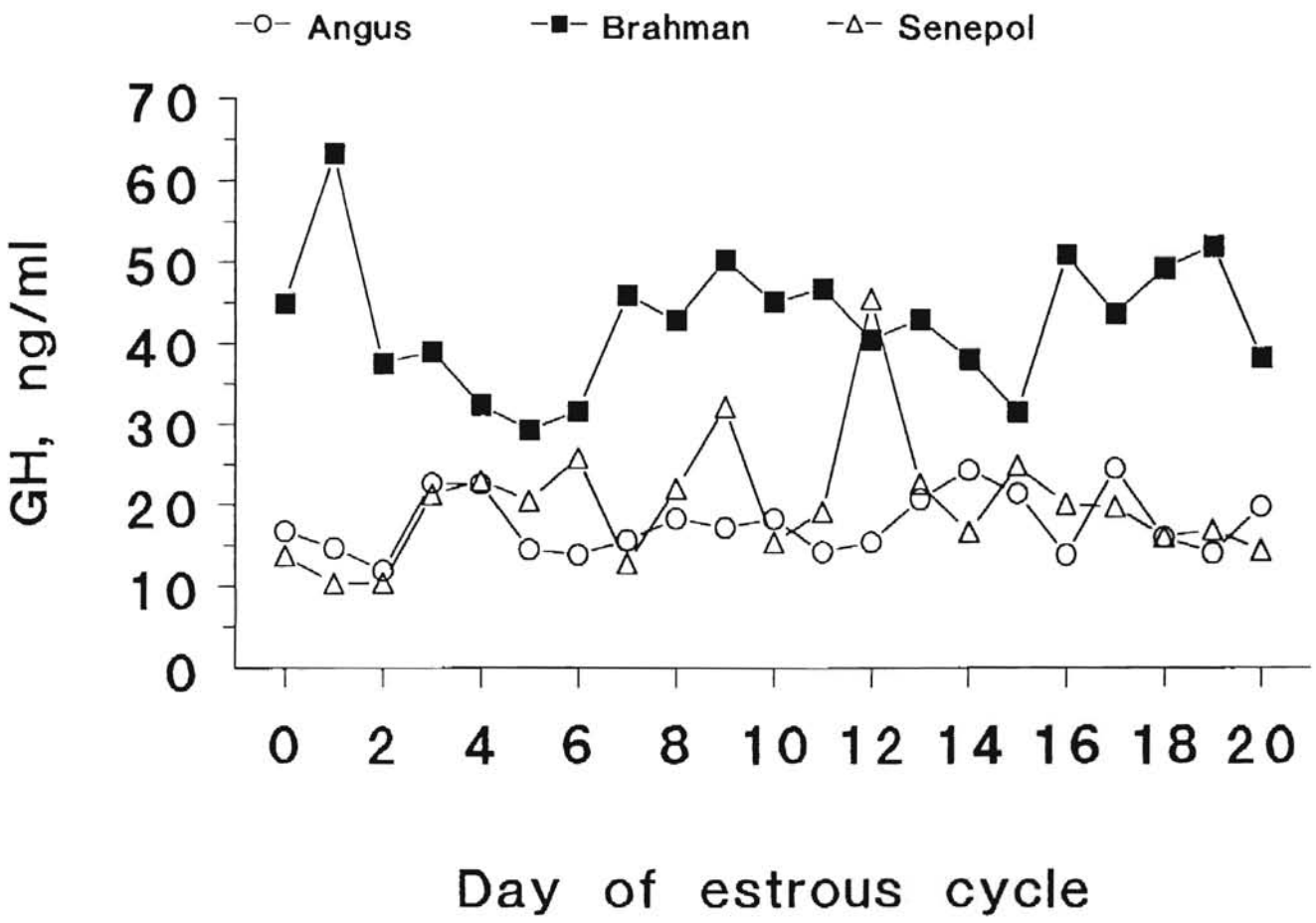

Fig. 11. Plasma concentrations of growth hormone (GH) in Angus, Brahman and Senepol cows as determined by RIA . Blood samples were collected daily. Standard errors averaged over the estrous cycle were 6.6, 7.4 and 6.6 for Angus, Brahman and Senepol cows, respectively.

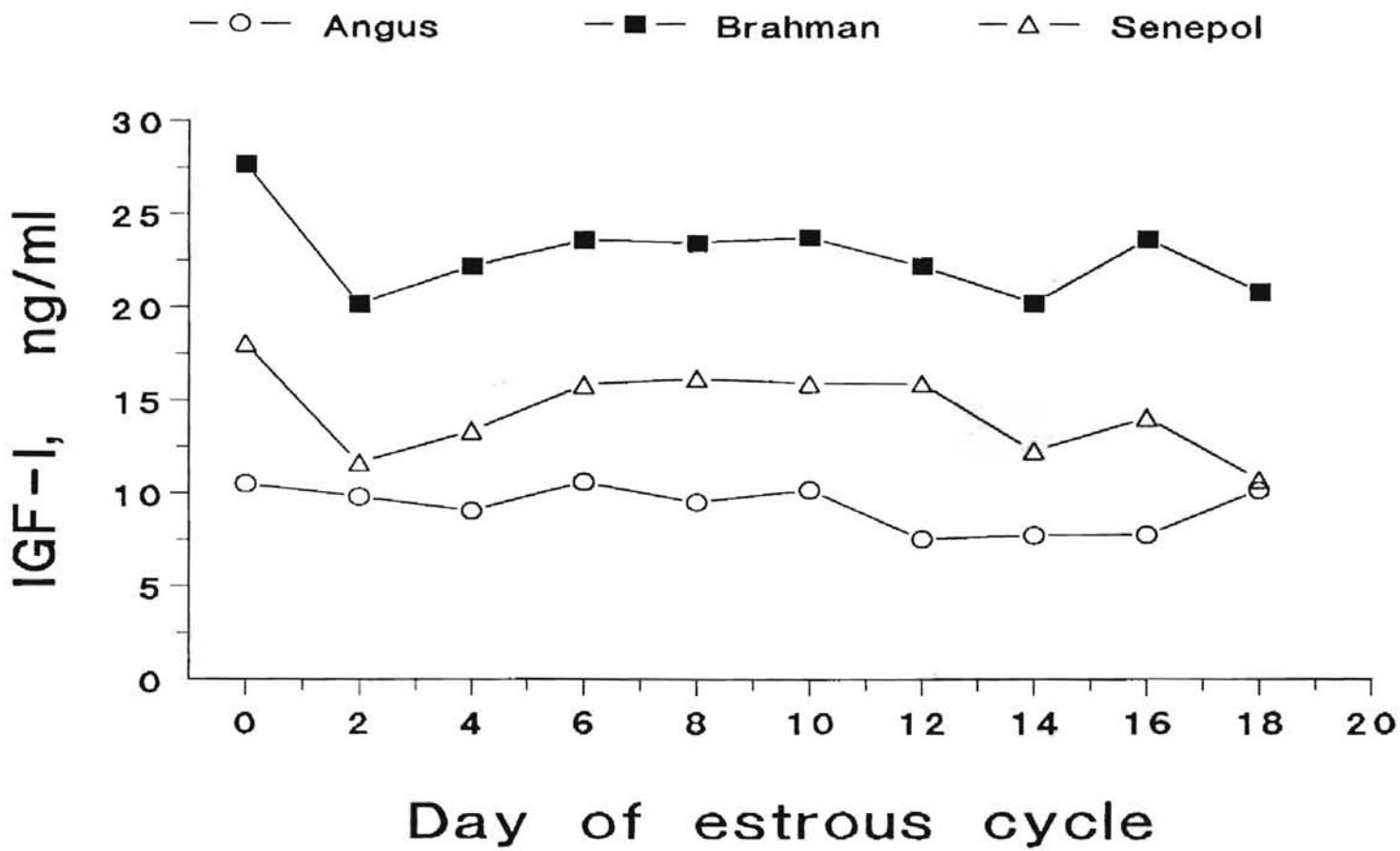

Fig. 12. Plasma IGF-I concentrations in Angus, Brahman and Senepol cows as determined by RIA. Blood samples were collected every other day. Standard errors averaged over the estrous cycle were 2.6, 2.7 and 2.4 for Angus, Brahman and Senepol cows, respectively.

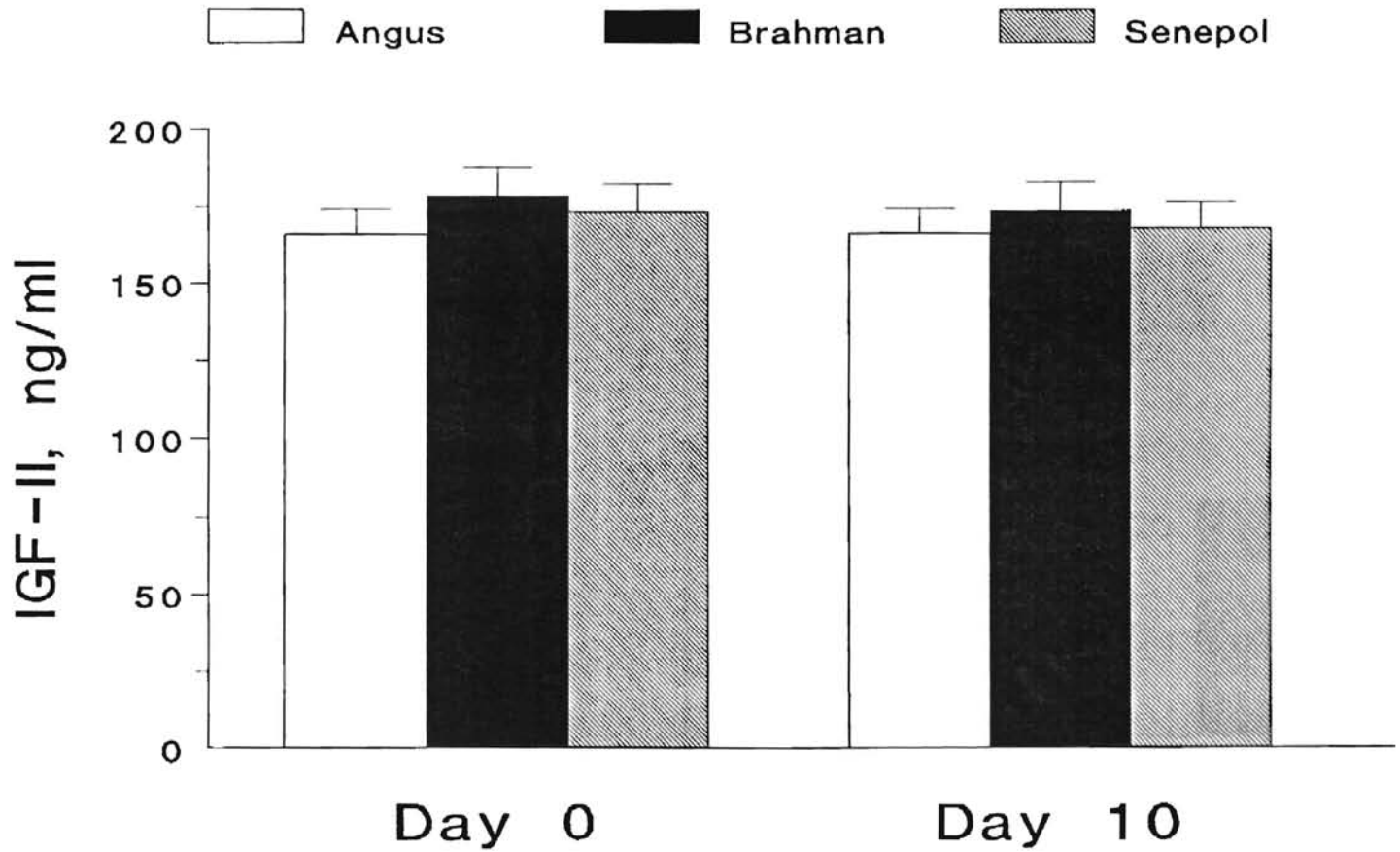

Fig. 13. Plasma IGF-II concentrations in Angus, Brahman and Senepol cows as determined by RIA. Blood samples were collected on day 0 and 10 of the estrous cycle.

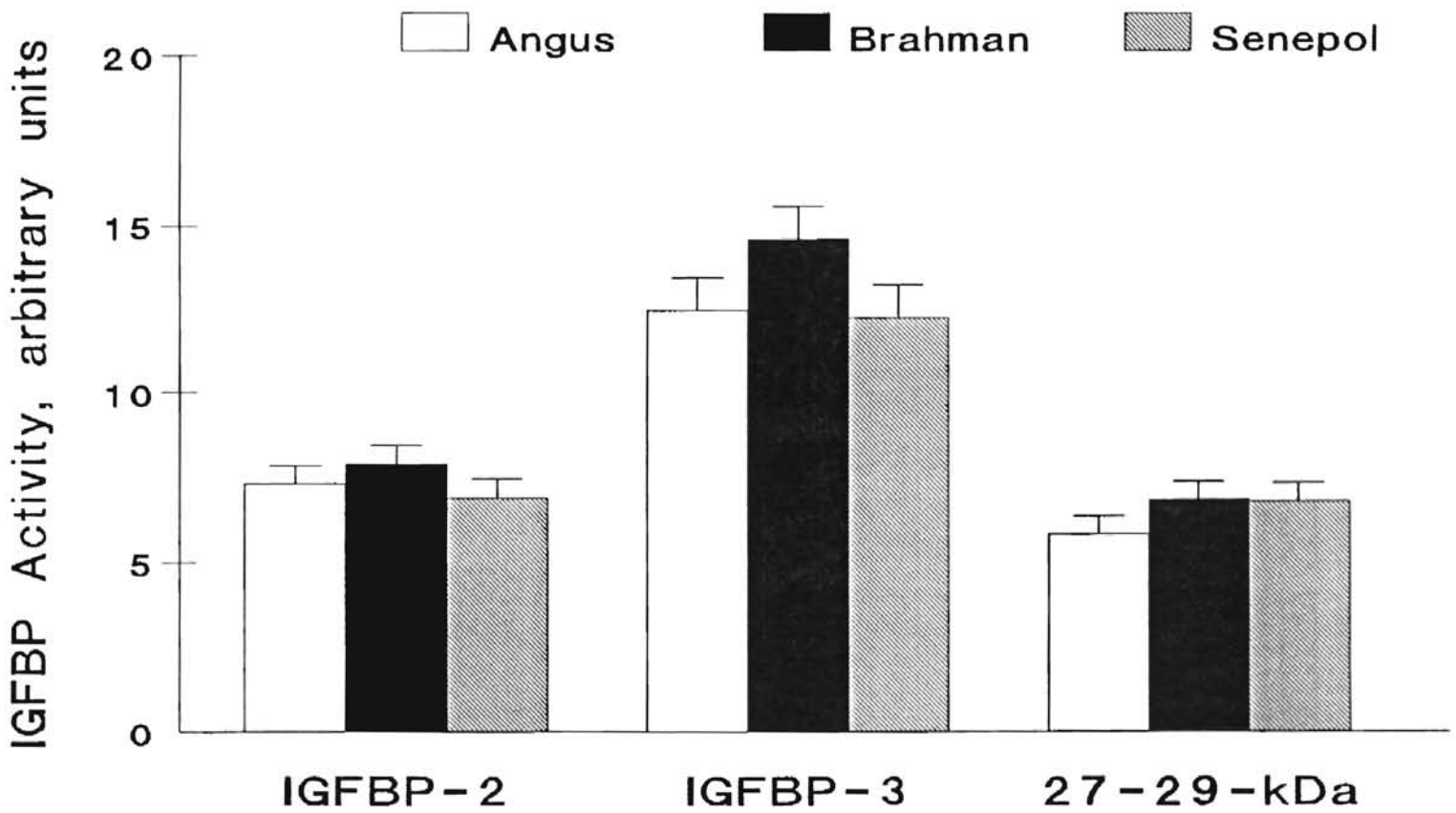

Fig. 14. Binding activity of IGFBP-2, IGFBP-3, and a 27-29-kDa IGFBP in Angus, Brahman and Senepol cows on day 10 of the estrous cycle.

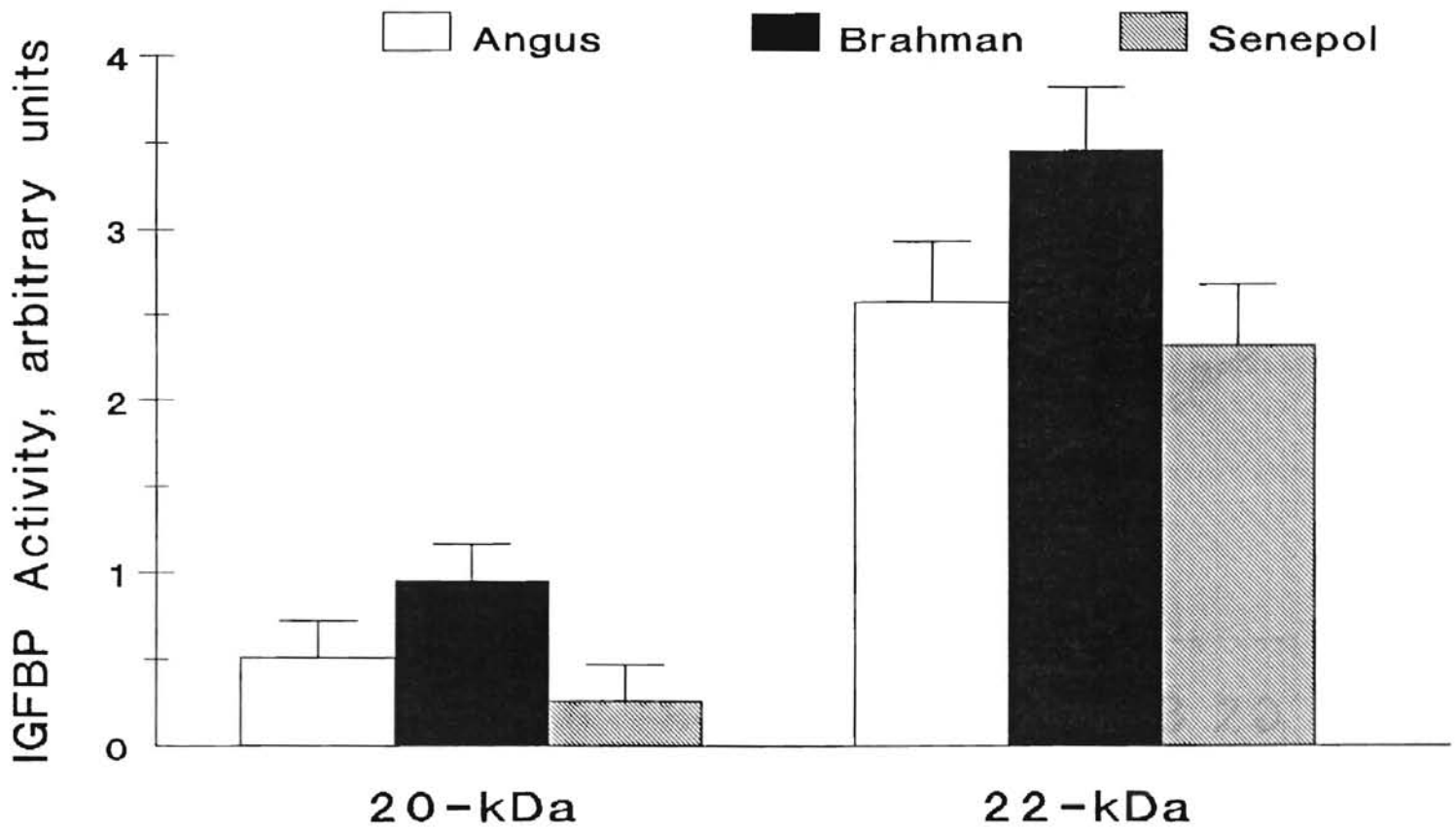

Fig. 15. Binding activity of a 20- and 22-kDa IGFBP in Angus, Brahman and Senepol cows on day 10 of the estrous cycle as determined by Ligand blotting.

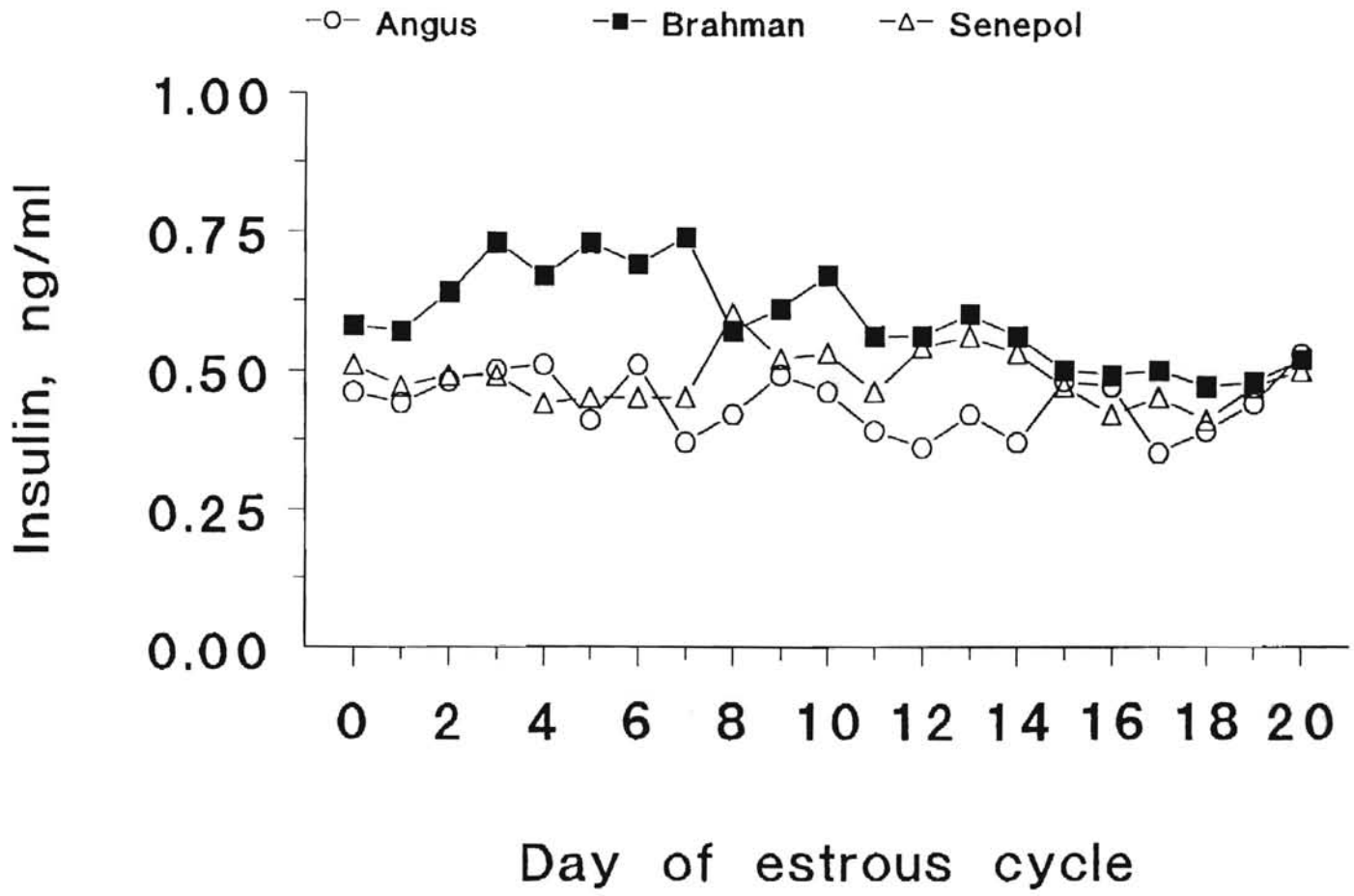

Fig. 16. Plasma concentrations of insulin in Angus, Brahman and Senepol cows as determined by RIA. Blood samples were collected daily. Standard errors averaged over the estrous cycle were 0.2, 0.3 and 0.2 for Angus, Brahman and Senepol cows, respectively.

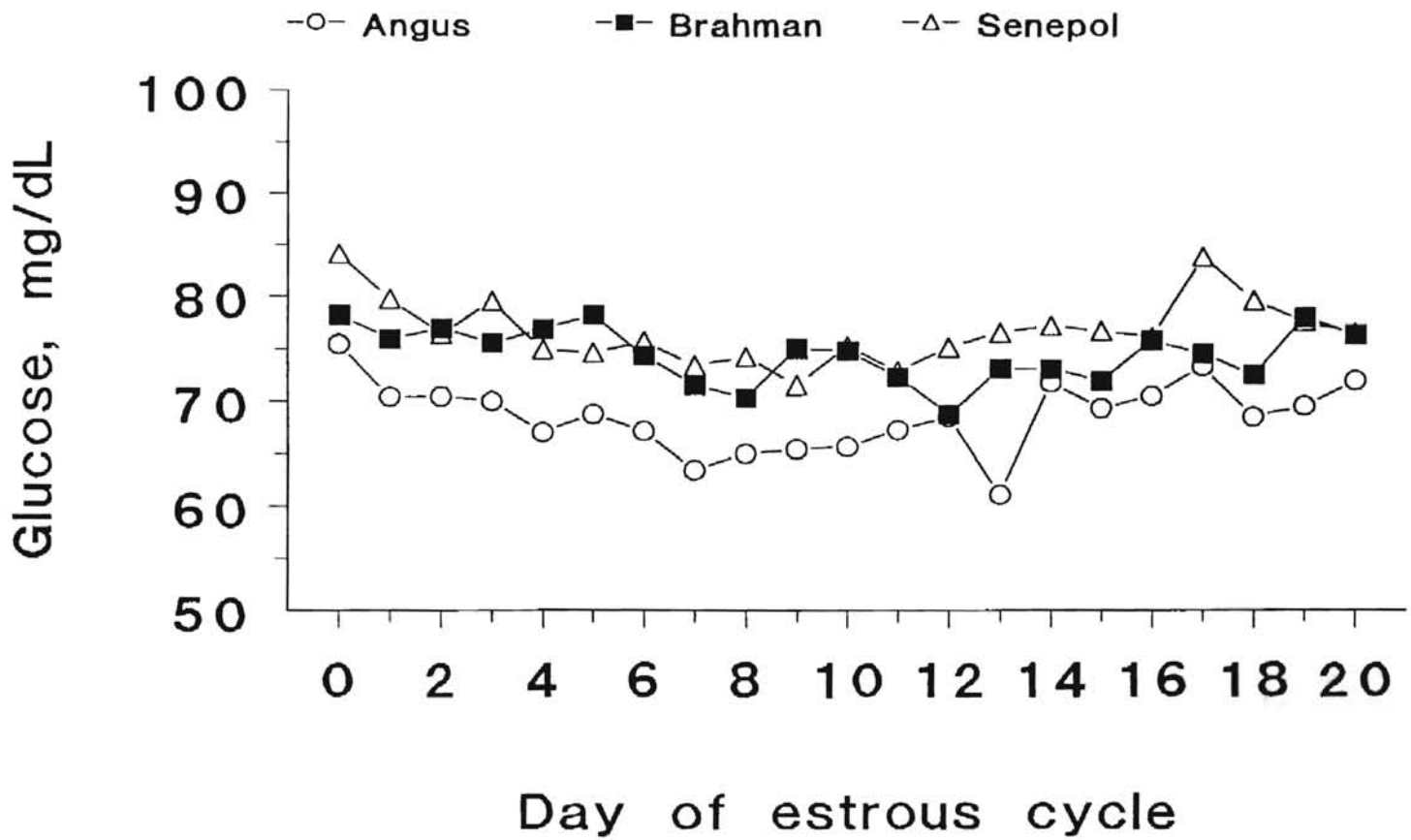

Fig. 17. Plasma concentrations of glucose in Angus, Brahman and Senepol cows as determined by automated calorimetric procedure. Blood samples were collected daily. Standard errors averaged over the estrous cycle were 3.2, 3.3 and 2.8 for Angus, Brahman and Senepol cows, respectively.

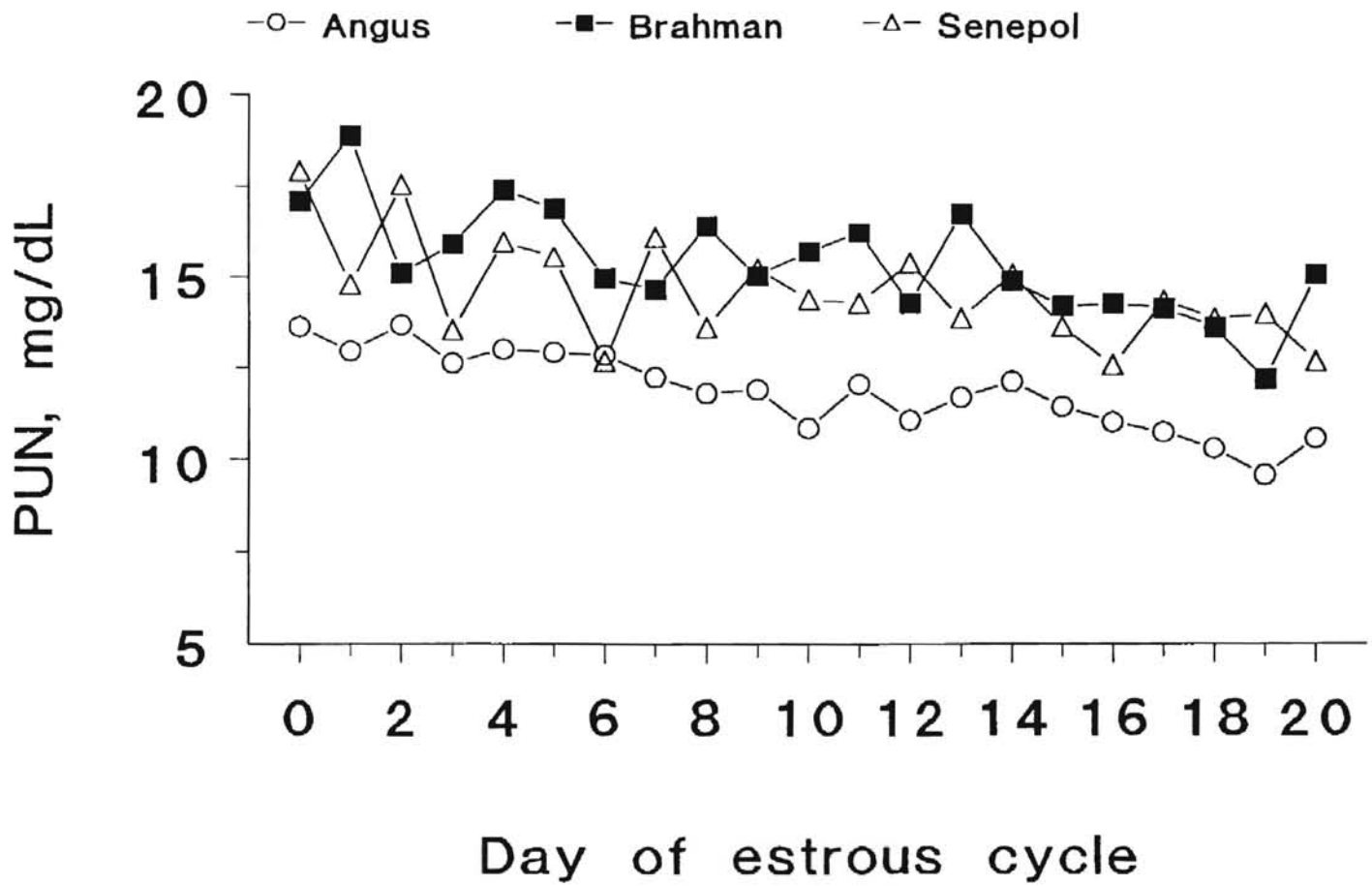

Fig. 18. Plasma concentrations of plasma urea nitrogen (PUN) in Angus, Brahman and Senepol cows as determined by automated calorimetric procedure. Blood samples were collected daily. Standard errors averaged over the estrous cycle were 0.9, 1.0 and 0.9 for Angus, Brahman and Senepol cows, respectively.

CHAPTER IV

SUMMARY AND CONCLUSIONS

For the first time since the application of ultrasound technology to the assessment of reproductive structures in cattle, ovarian dynamics in Bos taurus and Bos indicus cows were compared. Genotype differences in ovarian function were found in this study among Bos indicus (Brahman) cows, temperate Bos taurus (Angus) and tropically adapted Bos taurus (Senepol) cows maintained in a subtropical environment. The two breeds specifically adapted to the tropics (i.e., Brahman and Senepol) have a greater follicular activity than the non-adapted Angus cows as suggested by the greater number of follicles in all categories observed in the Brahman and Senepol cows. Whether greater number of follicles would represent an advantage in reproductive efficiency will require further research. The different pattern of endocrine secretion suggests a greater activity of the GH-IGF-I-insulin system in Brahman and Senepol cows as compared to the Angus cows. However, the activity of the gonadotropic axis is greater in Angus cows as suggested by the greater FSH concentrations found in that genotype, although no differences in LH secretion were found. The relative roles of those two systems in regulating reproductive performance in Bos taurus and Bos indicus cattle awaits additional studies. Also for the first time, the ovarian activity and endocrine pattern of Senepol cattle was studied.

Although, Senepol cattle are genotypically Bos taurus, their reproductive traits appear to be more similar to Brahman (Bos indicus) than to Angus (Bos taurus) cattle emphasizing the important role of the environment in the manifestation of genotypic characteristics.Senepol cows may represent a good source of germplasm that could prove beneficial in improving the efficiency of beef production in subtropical environments. More research needs to be conducted to ascertain the latter. And finally, this study was conducted in a subtropical environment in Florida during the summer, a complementary trial conducted during the Fall would help determining if there are genotype by environment interactions with Bos indicus (Brahman), temperate adapted Bos taurus (Angus) and tropically adapted Bos taurus (Senepol) cows.

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