Gonadal development and function in cattle

Kastelic, J.P.
EMBRAPA, CPPSE, Caixa Postal 339, São Carlos - SP, 13560-970, Brasil

INTRODUCTION - Reproduction is a key component of the cattle industry. The purpose of this article is to briefly review selected aspects of reproduction in cattle.

ESTRUS AND ESTROUS CYCLE - The estrous cycle in cattle is usually 21 days long (84% are 18 to 24 days; Allrich, 1993). Behavioral estrus lasts approximately 12 to 16 hours; ovulation occurs 10 to 15 hours after the end of estrus. True silent estrus is rare; most nonpregnant, anestrus dairy cows are cycling. However, dairy heifers often have a prolonged, (anovulatory) postpartum anestrus. For these cattle, increasing energy intake and/or short-term (7 to 10 days) treatment with progestins will hasten the onset of cyclicity.

The primary sign of estrus is a cow standing firm when mounted. Secondary signs of estrus include mounting, mucus discharge, swollen vulva, hyperactivity, and bellowing. The two principal causes of estrus-detection problems are missed estrus and estrus detection errors. Indicators of missed estrus include prolonged intervals from calving to breeding, prolonged intervals between breedings, more than 10 to 15% nonpregnant at pregnancy diagnosis, and less than 50% of potential estrus periods detected. Inadequate observation, slippery footing and adverse weather conditions can cause missed estrus. High progesterone concentrations (blood or milk) at breeding and interbreeding intervals less than 17 days or 25 to 35 days indicate errors in estrus detection. In some studies, up to 20% of cattle were not in estrus at breeding. Factors contributing to estrus detection errors include misinterpretation of signs of estrus, misinterpretation or misuse of estrus detection aids, and standing estrus in pregnant cows. Estrus detection can be improved by inducing estrus, improving the observer, increasing observation time, using estrus detection aids, and predicting the next estrus. Estrus detection aids include heat-mount detectors (mechanical or electronic; Stevenson et al., 1996) tail-head chalk or paint, pedometers and androgen-treated marker animals.

OVARIAN FOLLICULAR DEVELOPMENT - There are two principal ovarian structures, follicles and the corpus luteum. Ovarian follicular growth occurs in waves, the simultaneous development of several follicles on each ovary (Adams, 1994a; Kastelic, 1994). Remarkably, waves of follicular development were evident at 36 weeks of age in beef heifers, 20 weeks before puberty (Adams et al., 1994b). During the estrous cycle, the first wave begins around the day of ovulation, the second wave 8 to 10 days after ovulation and the third wave (if present) around 18 days after ovulation. One follicle per wave becomes dominant, grows for several days (maximum diameter, approximately 1.5 cm), and suppresses growth of the other (subordinate) follicles in the wave (maximum diameter, usually 1 cm). In the absence of luteal regression, the dominant follicle stops growing; in about 3 days a new wave starts and about 3 days later, regression of the previous dominant follicle is evident. The dominant follicle present at the time of luteal regression continues to grow and ovulates approximately 10 to 15 hours after the end of standing estrus. At the site of ovulation, a corpus luteum (CL) forms. The CL is maintained until calving (if pregnancy occurs) but in nonpregnant cattle, prostaglandin $F_{2\alpha}$ (PGF) is released from the uterus (about 16 days after estrus) and causes the CL to regress.

ESTRUS SYNCHRONIZATION - Synthetic PGF (and its analogues) are commonly used for estrus synchronization (Larson and Ball, 1992; Odde, 1990). Treatment with PGF will cause regression of a responsive CL. A CL may be responsive as early as 5 or 6 days after estrus, with responsiveness reaching a plateau approximately 10 days after estrus. In nonpregnant cattle treated late in the estrous cycle, the CL may already be regressing in response to endogenous PGF. The stage of follicular growth at the time of PGF treatment affects the interval from treatment to estrus and ovulation (Kastelic et al., 1990). With treatment 6 days after estrus, the dominant follicle of the first wave is still growing and the interval from treatment to ovulation is short (average, 3 days). Treatment 13 days after estrus requires a slightly longer interval to ovulation (average, 3.5 days) because the dominant follicle of the second wave is early in its growth phase. Treatment 9 days after estrus usually results in ovulation of the dominant follicle of the first wave but ovulation can occur from the dominant follicle of the second wave (average intervals to ovulation, 4 and 6 days, respectively; Kastelic et al., 1990; Kastelic and Ginther, 1991). Stage of estrous cycle also affects fertility; pregnancy rates are usually higher when cattle are treated with PGF after mid-cycle (e.g. after Day 12) compared to early in the cycle (e.g. Day 7 or 8). Fertility will usually be higher when cattle are bred after detection of estrus compared to breeding at a fixed time after PGF treatment.

There are many treatment regimens using PGF. In theory, approximately 70% of cycling cattle should have a CL responsive to PGF. Rectal palpation to identify cattle with a responsive CL should improve the response, but errors in palpation and missed estrus result in about 75% of treated cattle being detected in estrus (Gaines,

1994). Estrus detection and breeding for 5 days with PGF treatment on Day 5 to nonbred cattle works very well, but the period for estrus detection and breeding is approximately 10 days. With this regimen, failure to detect about 20% in estrus prior to treatment indicate problems with cyclicity and/or estrus detection. Two injections of PGF 11 days apart are commonly used, but increasing the interval to 14 days may improve fertility (Folman et al., 1990). One protocol (Ferguson and Galligan, 1993; Fuhrmann, 1993) utilizes PGF injections at 14-day intervals. The voluntary waiting period (minimum interval from calving to first breeding) is determined and all postpartum cattle 3 to 17 days prior to the voluntary waiting period are injected with PGF. Fourteen days later, these cows are again treated with PGF and bred according to signs of estrus activity. Any cattle not detected in estrus will receive PGF on the next treatment day (14 days later) and will be bred upon signs of estrus or at 80 hours after treatment if estrus has not been detected by that time. Pregnancy diagnosis is conducted as early as possible and nonpregnant cows are re-treated with PGF.

Treatment with GnRH causes the dominant follicle to ovulate (if it is growing or recently stopped growing) or to undergo atresia (if it is no longer viable), with emergence of a new follicular wave 2 or 3 days after treatment (Pursley et al., 1995). Treatment with GnRH, followed by PGF 6 or 7 days later, will result in 60 to 70% in estrus within 4 days after PGF treatment (Twagiramungu et al., 1995), with very few cattle in estrus before treatment with PGF. The synchrony of estrus (and in particular, the synchrony of ovulation) can be greatly increased by giving a second dose of GnRH 36 to 48 hours after PGF. Timed insemination 18 to 24 hours after the second GnRH treatment has resulted in acceptable fertility in cows, but poor fertility in heifers (Pursley et al., 1995).

Various progestins (progesterone-like compounds), including melengestrol acetate (MGA), have been utilized for estrus synchronization (Larson and Ball, 1992; Odde, 1990; Patterson et al., 1989). Progestin treatment for >14 days will synchronize estrus, but fertility will be reduced (only at the induced estrus) due to impairment of sperm transport, ovum fertilization and cleavage. Feeding 0.5 mg MGA/head/day for 14 days, followed by treatment with PGF 17 days after cessation of MGA (Patterson et al., 1989), results in well-synchronized estrus with good fertility, especially in cattle in at least moderate body condition (Yelich et al., 1995a). Furthermore, separating suckling calves from their dams for 48 h, starting 2 d after the last feeding of MGA, increased the percentage of 2 and 3 yr old dams conceiving early in the breeding season compared to a similar regimen without calf removal or untreated controls (Yelich et al., 1995b). Most cattle will be in estrus a few days after cessation of MGA; however, they are not bred until the estrus following PGF treatment. Pregnancy rates will be optimized by detecting estrus and breeding accordingly. Insemination at 72 hours after PGF treatment has resulted in good fertility in some herds but not others (King et al., 1994; Larson et al., 1996). Therefore, one approach is to inseminate all cattle at 72 hours after PGF and then either detect estrus and breed from 72 to 120 hours or expose cows to bulls starting at 96 hours (King et al., 1994).

Another regimen using MGA and PGF is to feed MGA for 7 to 9 days with PGF given on the last day. This approach results in somewhat lower fertility than giving PGF 17 days after MGA (Mauck et al., 1994). In this regimen, cattle which are started on MGA late in the estrous cycle undergo spontaneous luteal regression and develop a large ovarian follicle that elevates serum estradiol concentrations. However, giving 5 mg estradiol 17-beta and 100 mg progesterone (on the first day that MGA is fed) effectively synchronizes estrus and results in acceptable fertility (Kastelic et al., 1996a). The estradiol suppresses follicles present at the time of treatment, with synchronous emergence of a new follicular wave, on average, 4.3 days later (Bo et al., 1995). Progesterone is included to prevent an estrogen-induced LH surge in cattle without a functional CL.

There are two progesterone-containing devices (PRID and CIDR) used for estrous synchronization. These are placed in the vagina for 9 to 12 days. Luteal regression is achieved by treatment with an estrogen at the time the device is inserted and/or by treatment with PGF at the time of (or up to 2 days prior to) device removal. Most cattle will be in estrus 2 to 3 days after the device is removed, and fertility is generally acceptable.

Norgestomet is another progestin that is used in SyncroMate B and Crestar. An implant containing norgestomet is placed in the ear (similar to a growth implant) and an injection of norgestomet (3 mg) and estradiol valerate (5 mg) is given at the time of implant insertion. Implants are removed 9 days later. In most trials with SyncroMate B, >90% of treated cattle were detected in estrus, with pregnancy rates ranging from 33 to 68% (Odde, 1990). Fertility to timed insemination is generally acceptable (Odde, 1990). Many anestrus cattle will be detected in estrus following implant removal, but fertility will generally be lower than for cyclic cattle.

Ultrasound-guided transvaginal follicle aspiration has been utilized as a means of recovering oocytes for in vitro fertilization. This technique has also been used to ablate existing follicles to synchronize follicular growth, estrus and ovulation. In one study (Bergfelt et al., 1994), all follicles ≥5 mm in diameter were ablated and PGF injected 4 days later. This procedure synchronized

ovulation, particularly when a second dose of PGF was given (12 hours after the first dose).

SCROTAL/TESTICULAR THERMOREGULATION - Normal spermatogenesis in bulls is dependent upon maintenance of testicular temperature 2 to 6° C lower than body-core temperature. Many factors are involved in keeping the testes cool. A pendulous scrotum exposes the scrotal neck (facilitates heat loss) and allows the testes to move away from the body. The scrotal skin is usually thin and hairless, lacks subcutaneous fat, and has many blood vessels and lymphatics. The tunica dartos (smooth muscle under the scrotal skin) and the cremaster muscle (attached to the testis) can contract or relax to change the location of the testes. The testicular vascular cone (venous pampiniform plexus network surrounding the highly coiled testicular artery) is the site of heat loss, both by countercurrent exchange (transfer of heat from the artery to the vein) and radiation from the neck of the scrotum. Despite substantial changes in the anatomy of the scrotum, testes, and testicular vascular cone in bulls from 0.5 to 3 years of age, scrotal surface temperature was not significantly different (Cook et al., 1994).

Temperatures of the scrotum and testes have been investigated in three recent studies. In the first study (Kastelic et al., 1995), average temperatures (° C) at the top, middle and bottom of the testes were: 30.4, 29.8 and 28.8 (scrotal surface); 33.3, 33.0 and 32.9 (scrotal subcutaneous); and 34.3, 34.3 and 34.5 (intratesticular). Therefore, top-to-bottom temperature gradients were 1.6, 0.4 and -0.2° C (surface, subcutaneous and intratesticular, respectively). In the second study (Kastelic et al., 1996b), it was shown that the scrotum and testes have opposing temperature gradients which apparently complement one another, resulting in a relatively uniform intratesticular temperature. Furthermore, the scrotum substantially increased intratesticular temperature, but scrotal surface temperature was not significantly affected by the presence of a testis. These gradients may be due to vasculature. The scrotum is vascularized from top to bottom, but the testes are vascularized from the ventral aspect to the dorsal aspect (the testicular artery goes to the bottom of the testes and forms several branches that spread dorsally and laterally before entering the testes). In the third study (Kastelic et al., 1997), blood within the testicular artery had a similar temperature at the top of the testis as at the bottom, but was significantly cooler at the point of entry into the testicular parenchyma (intra-arterial temperatures 34.3, 33.4 and 31.7° C, respectively). Therefore, both the scrotum and testis are warmest at the origin of their vascular supply (top of scrotum, bottom of testis) with lower temperatures towards the opposite pole.

Infrared thermography is a noninvasive method of assessing scrotal surface temperature. The bull is restrained and a camera is held about 1 metre away (directed at the posterior aspect of the scrotum). The thermal pattern of the scrotum (thermogram) can be assessed visually or with computerized image analysis. Scrotal thermograms of bulls with normal scrotal thermoregulation had left-to-right symmetry and temperatures that were 4 to 6° C higher at the top of the scrotum than at the bottom (Coulter, 1988; Purohit et al., 1985). More random temperature patterns, often lacking left-to-right symmetry and having localized areas of increased temperature ("hot spots") were believed to be indicative of abnormal thermoregulation of the underlying testes or epididymides; these bulls usually had poor semen quality (Coulter, 1988; Purohit et al., 1985). However, not all bulls with poor semen quality had abnormal thermograms. The environmental conditions for thermography have been reported (Kastelic et al., 1996c). Thermography can be performed at any time of the day, but should be performed prior to feeding (or several hours thereafter) and at least 1 hour after rising. Moderate to cool ambient temperatures are preferable (range approximately 5 to 15° C) and the scrotum should be dry. Ejaculation (either spontaneous or electroejaculation) increases scrotal surface temperature over the cauda epididymides (Kastelic et al., 1996d).

Thermography has been used as an adjunct to the standard breeding soundness examination. In one study (Lunstra and Coulter, 1997), 30 yearling bulls, all judged satisfactory on a standard breeding soundness examination, were each exposed to approximately 18 heifers for a 45-day breeding period. For bulls with a SST pattern that was classified as normal or questionable, pregnancy rates 80 days after the end of the breeding season were similar (83 ± 3%, n = 13 versus 85 ± 4%, n = 9), but were higher (P<.01) than pregnancy rates for bulls with an abnormal SST pattern (68 ± 4%, n = 8).

INCREASED TESTICULAR TEMPERATURE - There are many potential causes of increased testicular temperature, including inflammation of the testes and/or scrotum, fever, prolonged recumbency (e.g. due to lameness), and increased ambient temperature (especially with high humidity). Many studies have been conducted in this area, with considerable variability in the results. However, several general conclusions can be made. When scrotal/testicular temperature is increased, sperm morphology usually appears normal for a short period, corresponding to transit time through the epididymis, and then begins to decline (Barth and Oko, 1989). In some studies (Kastelic et al., 1996e; Wildeus and Entwistle, 1983) spermatozoa that would have been in the epididymis at the time of scrotal heating were morphologically abnormal when collected soon after

heating. In another study (Vogler, 1991), changes in spermatozoa present in the epididymis at the time of scrotal insulation were manifest only after the spermatozoa were stressed (frozen and subsequently thawed). Sertoli and Leydig cell function seem adversely affected by heating, while germ cells are the most heat-sensitive cells in the testis (Waites and Setchell, 1990). All stages of spermatogenesis are susceptible to heating, with the extent of damage related to the extent and duration of the thermal insult (Waites and Setchell, 1990). Spermatocytes in meiotic prophase are killed by heat; spermatozoa that are more mature have metabolic and structural abnormalities (Setchell et al., 1971). Heating the testis usually decreases the proportion of progressively motile and live spermatozoa, and increases the incidence of morphologically abnormal spermatozoa, especially heads (Barth and Oko, 1989). Although there is considerable variation among bulls in the nature and proportion of defective spermatozoa, the order of appearance of specific defects is relatively consistent (Barth and Bowman, 1994; Vogler et al., 1993). Unless spermatogonia are affected, the interval from cessation of heating to restoration of normal spermatozoa in the ejaculate corresponds to the interval from the beginning of differentiation to ejaculation (Waites and Setchell, 1990). In most studies, sperm morphology had returned to pre-treatment values within approximately 6 weeks of the thermal insult. Very prolonged or severe heating of the testes will prolong the interval for recovery. However, even when sperm cell morphology has returned to normal, their utilization may result in decreased fertilization rates and an increased incidence of embryonic death (Burfening and Ulberg, 1968).

PUBERTY IN THE FEMALE - Nutrition has profound effects on the time of puberty, particularly in the heifer. The effects of nutrition on the onset of puberty have been reviewed (Schillo et al., 1992). Age at puberty is inversely related to the plane of nutrition; well-fed heifers reach puberty earlier and typically at a higher body weight than heifers on a poorer diet. Nutrition affects the release of LH, probably by modulation of GnRH release from the hypothalmus. Undernutrition appears to suppress the pulse frequency of LH needed for ovarian follicles to grow to the preovulatory stage (Schillo, 1992). Some of the effects of nutrition on puberty may be mediated through growth hormone (GH) and insulin-like growth factor-1 (IGF-1). Feed restriction increases GH, decreases IGF-1 and changes the relative proportions of IGF binding proteins (Benoit, et al., 1996). Immunization against growth hormone releasing factor decreases the number of large follicles present on the ovaries prior to puberty and delays puberty in heifers (Benoit, et al., 1996). Mean serum GH concentrations and GH pulse amplitude decreased dramatically just before puberty (Yelich et al, 1996).

Season has an effect on the onset of puberty in the beef heifer (Schillo et al., 1992a). Heifers born in the autumn reach puberty at younger ages than heifers born in the spring. Exposure to spring and summer temperatures and photoperiods from 6 to 12 months of age reduces age at puberty, regardless of season at birth (Schillo et al., 1992a). Photoperiod may be the major seasonal clue that influences onset of puberty in cattle. Perhaps melatonin is involved in transducing photoperiod stimuli into neuroendocrine signals that influence LH secretion (Schillo et al., 1992a).

There is a transient early rise in gonadotrophin secretion in young heifers (Evans et al., 1992). Significant opiodergic inhibition of LH secretion occurs only in very young heifers. A decrease in the opiod inhibition of LH secretion, particularly LH pulse amplitude, results in the early rise in LH secretion. Puberty is associated with sufficient LH release to cause final maturation of follicles and ovulation or luteinization. The first estrus is often silent and the first luteal phase may be short. Pregnancy rates improve almost 20% from the first to the third estrus (Byerley et al., 1987).

Increasing propionic acid in the rumen, influenced by both diet and ionophores, reduces age at puberty (Bagley, 1993). In one study, feeding monensin (200 mg/head/day) to Holstein heifers that weighed 217 or 330 kg reduced age at breeding by 24 and 15 days, and reduced age at calving by 61 and 36 days (Meinert et al., 1992).

Pasture alone often does not provide adequate energy for heifers to reach puberty at an early age, particularly if they have a large frame at maturity. In a study conducted in Brazil, when grazing Holstein heifers were supplemented with 0.3 kg cottonseed meal per day, 96 % had reached puberty by 18 months of age, compared to only 52% of unsupplemented heifers at the same age (Meirelles et al., 1994. However, excessive amounts of supplementation can increase fat deposition in the udder and reduce milk yield (Owens et al., 1993).

Continuous levels of adequate feed and growth are not essential. Fortunately, compensatory weight gains can overcome the effects of low levels of energy early in the feeding period. In one study (Penno et al., 1995), Holstein heifers (10 weeks of age) were fed to achieve weight gains of 0.8, 0.6 or 0.4 kg/day. The percentage of pubertal heifers at 11.5 months of age was 95%, 65% and 2% at liveweights of 268, 242 and 186 kg, respectively. When each group averaged 200 kg, they were then fed to gain 0.7 or 0.5 kg/day. At 15 months of age, only 82% of heifers initially fed the medium or low energy level followed by the low level had reached puberty, but 100% of all the other heifers had reached

puberty. However, nutrition, liveweight and time of puberty did not affect conception date or pregnancy rate.

Controlling parasites can also have an effect on puberty and reproductive performance. Beef heifers given ivermectin at 7 and 11 months of age and maintained on a marginal plane of nutrition had better weight gain, earlier puberty and higher pregnancy rate than non-treated heifers (Larson et al., 1995).

PUBERTY IN THE MALE - The onset of spermatogenesis in the male is a gradual process, occuring over an extended period. In a recent study in crossbred beef bulls (Evans et al., 1996), LH levels increased between 4 and 25 weeks of age. Circulating testosterone concentrations increased gradually from 6 to 35 weeks and then rapidly to 42 weeks. The number of cells in the advanced stages of spermatogenesis increased substantially between 15 and 45 weeks of age.

There is opiodergic inhibition of LH secretion in young bull calves; between 12 and 18 weeks, this inhibition decreased, resulting in increase LH pulse frequency (Evans et al., 1993). Consequently, there is an early transient rise in gonadotrophin secretion between 10 and 20 weeks of age in bull calves (Evans et al., 1995). In a study comparing bulls defined as early or late maturing (reached puberty at 41.9 and 48.3 weeks, respectively), serum concentrations of LH were significantly greater in early maturing than late-maturing bulls from 12 to 17 weeks of age, but there were no significant differences in concentrations of FSH or testosterone.

Puberty and semen traits were assessed in Holstein and Holstein x Gir bulls (Freneau et al., 1991). There was no significant difference in age at sexual maturity (12.2 months for each). Semen traits were not significantly different from 9 to 13 months of age; from 14 to 18 months, purebred bulls tended to have smaller ejaculates, more motile spermatozoa and higher sperm concentrations.

In one study with Friesan bulls (Alvarez et al., 1995), Holstein bulls were fed (from birth to 1 year of age) 100, 115, 122, or 130% of the recommended level of dietary energy. In these bulls, the average age at onset of sperm production was 327, 271, 314 and 338 days. Bulls fed the diet with 115% of recommended energy levels had the largest testicular volume, best libido and produced more and better quality spermatozoa. However, excessive levels of energy were detrimental. High levels of dietary energy fed to bulls after weaning can reduce both the quality and quantity of spermatozoa (Coulter and Kozub, 1984); some of these effects may be due to increased testicular temperature due to the deposition of fat in the neck of the scrotum (Coulter et al., 1997; Kastelic et al., 1996c).

GENETIC EFFECTS ON PUBERTY - Considerable genetic variation exists within and between breeds of beef cattle for age at puberty (Martin et al., 1992). In general, fast-growing breeds of larger mature size reach puberty later than do slow-growing breeds of smaller mature size; breeds with higher milk yield reach puberty faster than breeds with lower milk yield (Martin et al., 1992). Heterosis has a positive influence on puberty; crossbred heifers reach puberty at younger ages and heavier weights than purebreds. Scrotal circumference is an excellent indicator or age at puberty in yearling bulls. Larger scrotal circumference in bulls is related to early onset of puberty in female progeny (Martin et al., 1992).

BIBLIOGRAPHY

ADAMS, G.P. Control of ovarian follicular wave dynamics in cattle: Implications for synchronization and superstimulation. **Theriogenology**, v.41, p.19-24, 1994a.

ADAMS, G.P., EVANS, A.C.O., RAWLINGS, N.C. Follicular waves and circulating gonadotrophins in 8-month-old prepubertal heifers. **J. Reprod. Fertil.**, v.100, p.27-33, 1994b.

ALLRICH, R.D. Estrous behaviour and detection in cattle. The Vet. Clinics of North America: **Food Animal Practice**, v.9, p.249-262, 1993.

ALVAREZ, J.L., ZALDIVAR, V., BOADO, A., et al. Influence of the energy and protein levels in rations on the onset of puberty and semen quality in male Holstein. **Revista de Salud. Animal**, v.17, p.189-200, 1995.

BAGLEY, C.P. Nutritional management of replacement beef heifers: a review. **J. Anim. Sci.**, v.71, p.3155-3163, 1993.

BARTH, A.D., OKO, R.J. Abnormal morphology of bovine spermatozoa. Ames: Iowa State University Press, 1989, p. 139.

BARTH, A.D., BOWMAN, P.A. The sequential appearance of sperm abnormalities after scrotal insulation or dexamethasone treatment in bulls. **Can. Vet. J.**, v.35, p.93-102, 1994.

BENOIT, A.M., SWANCHARA, K., SCHOPPEE, P., et al. Insulin-like growth factor-I (IGF-I) and IGF binding proteins: potential mediators of the influence of nutrition on ovarian function in the heifer and gilt. **Reprod. Dom. Anim.**, v.31, p.549-553, 1996.

BERGFELT, D.R., LIGHTFOOT, K.C., ADAMS, G.P. Ovarian synchronization following ultrasound-guided transvaginal follicle ablation in heifers. **Theriogenology**, v.42, p.895-907, 1994.

BO, G.A., ADAMS, G.P., PIERSON, R.A., et al. Exogenous control of ovarian follicular wave emergence in cattle. **Theriogenology**, v.43, p.31-40, 1995.

BURFENING, P.J., ULBERG, L.C. Embryonic survival subsequent to culture of rabbit spermatozoa at 38° and 40°. **J. Reprod. Fert.**, v.15, p. 87-92, 1968.

BYERLEY, D.J., STAIGMILLER, R.B., BERARDINELLI, J.G., et al. Pregnancy rates of beef heifers bred either on puberal or third estrus. **J. Anim. Sci.** v.65, p.645-650, 1987.

COOK, R.B., COULTER, G.H., KASTELIC, J.P. The testicular vascular cone, scrotal thermoregulation, and their relationship to sperm production and seminal quality in beef bulls. **Theriogenology**, v.41, p. 653-671, 1994.

COULTER, G.H., KOZUB, G.C. Testicular development, epididymal sperm reserves and seminal quality in two-year-old Hereford and Angus bulls: effects of two levels of dietary energy. **J. Anim. Sci.**, v.59, p.432-440, 1984.

COULTER, G.H. Thermography of bull testes. Proc., 12th In: INTERNATIONAL CONFERENCE ARTIFICIAL INSEMINATION ANIMAL REPRODUCTION, 1988, Milwaukee, WI. **Proceedings...** Columbia, MO: National Association of Animal Breeders, 1988, p.58-63.

COULTER, G.H., COOK, R.B., KASTELIC, J.P. Effects of dietary energy on scrotal surface temperature, seminal quality, and sperm production in young beef bulls. **J. Anim. Sci.**, v.75, p.1048-1052, 1997.

EVANS, A.C.O., CURRIE, W.D., RAWLINGS, N.C. Effects of naloxone on circulating gonadotrophin concentrations in prepubertal heifers. **J. Reprod. Fertil.**, v.96, p.847-855, 1992.

EVANS, A.C.O., CURRIE, W.D., RAWLINGS, N.C. Opioidergic regulation of gonadotrophin secretion in the early prepubertal bull calf. **J. Reprod. Fertil.**, v.99, p.45-51, 1993.

EVANS, A.C.O., DAVIES, F.J., NASSER, L.F., et al. Differences in early patterns of gonadotrophin secretion between early and late maturing bulls, and changes in semen characteristics at puberty. **Theriogenology**, v.43, p.569-578, 1995.

EVANS, A.C.O., PIERSON, R.A., GARCIA, A., et al. Changes in circulating hormone concentrations, testes histology and testes ultrasonography during sexual maturation in beef bulls. **Theriogenology**, v.46, p.345-357, 1996.

FERGUSON, J.D., GALLIGAN, D.T. Prostaglandin synchronization programs in dairy herds. Part I. **The Compendium on Continuing Education for the Practicing Veterinarian** v.15, p.646-655, 1993.

FOLMAN, Y., KAIM, M., HERZ, Z. et al. Comparison of methods for the synchronization of estrous cycles in dairy cows. 2. Effects of progesterone and parity on conception. **J. Dairy Sci.**, v.73, p.2817-2825, 1990.

FRENEAU, G.E., GUIMARAES, J.D., FONSECA, V.O., et al. Puberty and semen traits in purebred Holstein and Holstein X Gir F1 bulls, aged 7-18 months and reared at Ibia, Minas Gerais. In: CONGRESSO BRASILEIRO DE REPRODUÇÃO ANIMAL, 9., 1991, Belo Horizonte. *Anais...* Belo Horizonte : CBRA, 1991., v.2, p.424.

FUHRMANN, T. Taking control of reproduction in dairy herds. In: THERIOGENOLOGY ANNUAL MEETING, 1993, Jacksonville, FL. *Proceedings...* Hastings, NE: Society for Theriogenology, 1993, p.97-106.

GAINES, J.D. The use of gonadotropin releasing hormone and prostaglandin for programmed breeding of dairy cattle. In: In: THERIOGENOLOGY ANNUAL MEETING, 1994, Kansas City, MO. *Proceedings...*Hastings, NE: Society for Theriogenology, 1994, p.108-120.

KASTELIC, J.P., KNOPF, L., GINTHER, O.J. Effect of day of prostaglandin $F_{2\alpha}$ treatment on selection and development of the ovulatory follicle in heifers. **Anim. Reprod. Sci.**, v. 23, p.169-180, 1990.

KASTELIC, J.P., GINTHER, O.J. Factors affecting the origin of the ovulatory follicle in heifers with induced luteolysis. **Anim. Reprod. Sci.**, v.26, p.13-24, 1991.

KASTELIC, J.P. Ovarian follicular development in cattle. **Vet. Med.**, v.89, p.64-71, 1994.

KASTELIC, J.P., COULTER, G.H., COOK, R.B. Scrotal surface, subcutaneous, intratesticular, and intraepididymal temperatures in bulls. **Theriogenology**, v.44, p.147-152, 1995.

KASTELIC, J.P., McCARTNEY, D.H., GARCIA, A., et al. Estrus synchronization in cattle using estradiol, melengestrol acetate and PGF. **Theriogenology**, v.46, p.1295-1304, 1996a.

KASTELIC, J.P., COOK, R.B., COULTER, G.H. Contribution of the scrotum and testes to scrotal/testicular thermoregulation in bulls and rams. **J. Reprod. Fertil.**, v.108, p.81-85, 1996b.

KASTELIC, J.P., COOK, R.B., COULTER, G.H., et al. Environmental factors affecting measurement of bovine scrotal surface temperature with infrared thermography. **Anim. Reprod. Sci.**, v.41, p.153-159, 1996c.

KASTELIC, J.P., COOK, R.B., COULTER, G.H., et al. Ejaculation increases scrotal surface temperature in bulls with intact epididymides. **Theriogenology**, v.46, p.889-892, 1996d.

KASTELIC, J.P., COOK, R.B., COULTER, G.H., et al. Insulating the scrotal neck affects semen quality and scrotal/testicular temperatures in the bull. **Theriogenology**, v.45, p.935-942, 1996e.

KASTELIC, J.P., COOK, R.B., COULTER, G.H. Contribution of the scrotum, testes, and testicular artery to scrotal/testicular thermoregulation in bulls at two ambient temperatures. **Anim. Reprod. Sci.**, v.45, p.255-261, 1997.

KING, M.E., DANIEL, M.J., TEAGUE, L.D., et al. Comparison of timed insemination with insemination at estrus following synchronization of estrus with a MGA-prostaglandin system in beef heifers. **Theriogenology**, v.42, p.79-88, 1994.

LARSON, L.L., BALL, P.J.H. Regulation of estrous cycles in dairy cattle: a review. **Theriogenology**, v.38, p.255-267, 1992.

LARSON, R.L., CORAH, L.R., SPIRE, M.F. et al. Effect of treatment with ivermectin on reproductive performance of yearling beef heifers. **Theriogenology**, v.44, p.189-197, 1995.

LARSON, R.L., CORAH, L.R., PETERS, C.W. Synchronization of estrus in yearling beef heifers with the melengestrol acetate/prostaglandin $F_{2\alpha}$ system: efficiency of timed insemination 72 hours after prostaglandin treatment. **Theriogenology**, v.45, p.851-863, 1996.

LUNSTRA, D.D., COULTER, G.H. Relationship between scrotal infrared temperature patterns and natural-mating fertility in beef bulls. **J. Anim. Sci.**, v.75, p.767-774, 1997.

MARTIN, L.C., BRINKS, J.S., BOURDON, R.M., et al. Genetic effects on beef heifer puberty and subsequent reproduction. **J. Anim. Sci.**, v.70, p.4008-4017, 1992.

MAUCK, H.S., KING, M.E., HOLLAND, M.D., et al. Comparison of two MGA-$PGF_{2\alpha}$ systems for synchronization of estrus in beef heifers. **Theriogenology**, v.42, p.951-961.

MEINERT, R.A., YANG, C.M.J., HEINRICHS, A.J., et al. Effect of monensin on growth, reproductive performance, and estimated body composition in Holstein heifers. **J. Dairy Sci.**, v.75, p.257-261, 1992.

MEIRELLES, C.F., ABDALLA, A.L., VITTI, D.M.S.S. The effect of feed supplement on the onset of puberty in Brazilian dairy heifers. **Scientia Agricola**, v.51, p.374-380, 1994.

ODDE, K.G. A review of synchronization of estrus in postpartum cattle. **J. Anim. Sci.**, v.68, p.817-830, 1990.

OWENS, F.N., DUBESKI, P., HANSON, C.F. Factors that alter the growth and development of ruminants. **J. Anim. Sci.**, v.71, p.3138-3150, 1993.

PATTERSON, D.J., KIRACOFE, G.H., STEVENSON, J.S., et al. Control of the bovine estrous cycle with melengestrol acetate (MGA): a review. **J. Anim. Sci.**, v.67, p.1895-1906, 1989.

PENNO, J.W., MACMILLAN, K.L., BRYANT, A.M. Effect of level nutrition on age of puberty and reproductive performance of Friesian heifers. **Proc. New Zeal. Soc. Anim. Prod.**, v.55, p.79-81, 1995.

PUROHIT, R.C, HUDSON, R.S., RIDDELL, M.G. Thermography of the bovine scrotum. **Am. J. Vet. Res.**, v.46, p.2388-2392, 1985.

PURSLEY, JR, MEE, MO, WILTBANK MC. Synchronization of ovulation in dairy cows using PGF2a and GnRH. **Theriogenology**, v.44, p.915-923, 1995.

SCHILLO, K.K. Effects of dietary energy on control of luteinizing hormone secretion in cattle and sheep. **J. Anim. Sci.**, v.70, p.1271-1282, 1992.

SCHILLO, K.K., HALL, J.B., HILEMAN, S.M. Effects of nutrition and season on the onset of puberty in the beef heifer. **J. Anim. Sci.**, v.70, p.3994-4005, 1992.

SETCHELL, B.P., VOGLMAYR, J.K., HINKS, N.T. The effect of local heating on the flow and composition of rete testis fluid in the conscious ram. **J. Reprod. Fertil.**, v.24, p.81-90, 1971.

STEVENSON, J.S., SMITH, M.W., JAEGER, J.R. et al. Detection of estrus by visual observation and radiotelemetry in peripubertal, estrus-synchronized beef heifers. **J. Anim. Sci.**, v.74, p.729-735, 1996.

TWAGIRAMUNGU, H., GUILBAULT, L.A., DUFOUR, J.J. Synchronization of ovarian follicular waves with a gonadotropin-releasing hormone agonist to increase the precision of estrus in cattle: a review. **J. Anim. Sci.**, v.73, p.3141-3151, 1995.

VOGLER, C.J., SAACKE, R.G., BAME, J.H., et al. Effects of scrotal insulation on viability characteristics of cryopreserved bovine semen. **J. Dairy Sci.**, v.74, p.3827-3835, 1991.

VOGLER, C.J., BAME, J.H., DEJARNETTE, J.M., et al. Effects of elevated testicular temperature on morphology characteristics of ejaculated spermatozoa in the bovine. **Theriogenology**, v.40, p.1207-1219, 1993.

WAITES, G.M.H., SETCHELL, B.P. Physiology of the Mammalian Testis 4.ed In: LAMMING, G.E. *Reproduction in the Male*,. Churchill Livingstone, Edinburgh: Marshall's Physiology of Reproduction, 1990, v.2,p.1-105

WILDEUS, S., ENTWISTLE, K.W. Spermiogram and sperm reserves in hybrid Bos indicus x Bos taurus bulls after scrotal insulation. **J. Reprod. Fertil.**, v.69, p.711-716, 1983.

YELICH, J.V., MAUCK, H.S., HOLLAND, M.D., et al. Synchronization of estrus in suckled postpartum beef cows with melengestrol acetate and $PGF_{2\alpha}$. **Theriogenology**, v.43, p.389-400, 1995a.

YELICH, J.V., HOLLAND, M.D., SCHUTZ, D.N., et al. Synchronization of estrus in suckled postpartum beef cows with melengestrol acetate, 48-hour calf removal and $PGF_{2\alpha}$. **Theriogenology**, v.43, p.389-400, 1995b.

YELICH, J.V., WETTEMANN, R.P., MARSTON, T.T., et al. Luteinizing hormone, growth hormone, insulin-like growth factor-I, insulin and metabolites before puberty in heifers fed to gain at two rates. **Dom. Anim. Endocrin.**, v.13, p.325-338, 1996.