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Gonadotropin and gonado-hormone concetrations in the pygmy goat during transition from anestrus to breeding activity

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Abstract

Blood samples obtained from two mature and two prepubertal female pygmy goats during anestrus up to four days after first overt estrus were assayed for gonadotropins and gonado-hormones by radioimmunoassay (RIA) techniques. The prepubertal goats were aged 211 and 214 days respectively. All animals showed behavioral and endocrine changes that were characteristic of the establishment of normal estrus cycles between the 18th and 30th of November. The two prepubertal does showed first overt estrus at the age of 248 and 251 days, respectively. From progesterone concentration profiles, all animals were observed to have had a presumed ovulation without overt estrus at an average of 19.2 $\pm$ 0.5 days before the first behavioral estrus. LH and FSH levels fluctuated randomly throughout anestrus; a two-to three fold increase in their mean values was apparent 5 days before the pre-ovulatory surge prior to the first presumed ovulation. The pattern of their secretion during the interval from first presumed ovulation to first behavioral estrus was similar to that observed during anestrus; both were elevated on the day of first behavioral estrus. Progesterone levels were low during anestrus; their first elevation (from 0.5 $\pm$ 0.08 to 2.3 + 0.4 ng/ml) occorred 2 days before the first presumed ovulation (day-23). During the interval between the first presumed ovulation and first overt estrus, progesterone concentrations and pattern of secretion were characteristic of that observed during the oormal cycle. Estradiol concentrations were varied during anestrus and the interval from first presumed ovulation to first overt estrus. Levels of estradiol were observed to be elevated a day before the LH/FSH preovulatory surges of the first presumed ovulation and the first overt estrus, respectively.

Key words: Pygmy goats, anestrus, puberty, breeding activity, gonodotrophins, gonodo - hormones.

Introduction

The main factors limiting continued productivity in seasonal breeders during the periods of anestrus are the failure to exhibit estrus and the continued absence of ovulation illustrated by relatively regressed ovaries that lack functional corpora lutea.

The ovarian quiescence and regression typical of anestrus cannot be attributed to a deficiency in gonadotropin secretion since the pituitary concentrations of LH and FSH are similar in ewes and ewe-lambs during anestrus and the breeding season (Roche et al., 1970; Foster and Ryan, 1979). Rather it is due to change in the responsiveness of the hypothalamo-pituitary unit toward gonado-hormones feedback mechanisms (Legan and Karsch, 1979; Ducharme et al., 1979; Keisler et al., 1987; Kinder et al., 1987; Ozsar et al., 1990).

The evaluation of estradiol, progesterone and LH by Yuthasastrakosol et al. (1975) and of FSH, LH, prolactin and progesterone by Walton et al. (1977) has shown how these hormones interrelate in intact ewes during anestrus and during the transition from anestrus to the establishment of regular estrous cycles. There is no such information on the goat. Therefore, this study was conducted with the aim of trying to establish the interrelationships of various hormones during the transition from anestrus to resumption of breeding activity and attainment of puberty in the Pygmy goat.

Materials and methods

Animals

Four female pygmy goats (2 mature and 2 prepubertal) were bled daily by jugular venipuncture throughout anestrus beginning June 15th, 1976 up to four days after the first overt estrus. The two prepubertal goats were 211 and 214 days of age at the initiation of sampling. The blood samples were kept refrigerated at 4°C for 24 hrs. They were then centrifuged and plasma was aspirated from the clot and kept at -20°C until assayed.

Homone Assays

The determination of various hormone concentrations was performed by radioimmunoassay (RIA). Details of individual hormone RIA procedures have already been reported for progesterone and estrogen by Yulthasastrakosol et al., (1975) for LH by Simaraks (1978) and for FSH by Cheng (1976). Individual descriptions of each methodology follows below.

Progesterone

Serum progesterone concentrations were determined by use of a method previously described by Yuthasastrakosol et al., 1975. A highly specific antiserum (Pool #337) was generously provided by Dr. G. Niswender (Colorado State University). The antiserum was prepared by immunization of a rabbit with progesterone - 6 - hemisuccinate - BSA.

The percentage cross-reaction of the antiserum with various steroids have been described by Yulthasastrakosol et al., (1975) and 5 -pregnane-3, 20-dione is the only steroid that had significant cross-reactivity (>3%).

A routine determination of progesterone concentration in a pool sample from does in various reproductive states was used to determine inter-assay and intra-assay coefficients of variations. The average progesterone concentration in the pool sample was 3.8 ± 0.08 ng/ml determined in 12 separate assays. This yielded inter-assay and intra-assay coefficients of variations of 16.1% and 9.6%, respectively. The mean percentage recovery of ^{3}H-progesterone added to serum samples was 77.7 ± 0.6 (n = 11) and was utilized to correct the results for procedural losses.

Estrogen

Serum estrogen concentrations were evaluated by a radioimmunoassay previously described by Yu et al., (1974). This methodology omits column chromatography and thus measures total estrogens. The anti-estradiol-17b, BSA (#029-14) used in this assay was obtained from Dr. B. Caldwell (Yale University). The antiserum was prepared by immunizing sheep with estradiol - 17b, 17 - hemisuccinate - BSA. the specificity of this antiserum had been extensively characterized by Wu and Lundy (1971). Its cross-reactivity to various estrogens was found to be 100% for estradiol - 17b, 63.7% for estrone and 5.1% for estradiol - 17a.

The mean percentage of recovery when ^{3}H - estradiol was added to serum samples was 63.8 ± 1.8 (n = 8). A routine estrogen measurement on a pool sample obtained from does in various reproductive states was utilized to determine inter-assay and intra-assay coefficients of variations. In 14 separate duplicate determinations, the average estrogen concentration in the pool sample was 27.4 ± 0.9 pg/ml with inter-assay and intra-assay coefficients of variations of 16.0% and 11.1% respectively.

The standards used in the assays of both progesterone and estrogen were obtained from Mann Research laboratories, Orangeburg, new York and were used without further chromatographic treatment.

Lutcinizing hormone (LH)

A double antibody RIA was used to measure LH concentrations. Details of the procedure have been previously described by Simaraks (1978). Anti-ovine LH serum (GDN #15) supplied by Dr. G. Niswender (Colorado state University) was used in the assay. Purified ovine LH (LER-1056-02) was labelled with ^{125}Iodine (Cambridge Nuclear Corporation) by a modified procedure that has been described by Sanford (1974) and Yuthasastrakosol et al., (1975) and was previously used by Muduuli (1978) to assay LH in male pygmy goats. LH values are expressed as ng/ml of NIH-LH-S14 standard. By using LH values obtained in 12 separate duplicate assays carried out on a pool sample whose average LH concentration was 2.28 ± 0.4 ng/ml, it was found that the inter-assay and intra-assay coefficients of variations were 11.4% and 13.7%, respectively. The lowest detectable levels using serially diluted pool serum was 0.2 ng/ml, samples yielding values lower than 0.2 ng/ml were assigned that value for statistical purposes.

Follicle-stimulating hormone (FSH)

Serum FSH levels were evaluated using a procedure that employed an antibody developed against bovine FSH (Cheng, 1976). This antibody has been characterised and used to assay ovine FSH (Simaraks (1978). The same antibody was used in this study for the assay of caprine FSH. The antibody has been shown to exhibit parallel curves for ovine FSH preparations (Simaraks, 1978). Since Muduuli (1978) showed that parallelism existed between sheep and goat preparations it was assumed that the evaluation of FSH in caprine serum was valid if this antibody was used. Hence, its use was made in this study without further parallelism tests. Purified bovine FSH was labelled with ^{125} Iodine (Cambridge Nuclear Corporation) by a modification of the method of Greenwood et al., (1963). The intra-assay and inter-assay coefficients of variation were determined using procedures described above. In 12 separate assays using pooled serum, the mean FSH concentration was found to be 26.1 ± 0.8 ng/ml. The inter-assay and intra-assay coefficients of variations were estimated to be 9.5% and 11.9%, respectively (n = 11). The minimum detectable level of FSH at 95% of initial binding (B/Bo) was 0.7 ng/ml. Values of FSH are expressed as ng/ml of NIH-FSH-S12 (ovine).

Results and discussion

Onset of breeding activity and/or puberty

All the animals showed behavioural and endocrine changes that were characteristic of the establishment of normal estrus cycles between the 18th and 30th of November. The interval from first presumed ovulation (as indicated by progesterone profiles) to first behaviourial estrus of the breeding season was 18, 20, 19 and 20 days for goat #'s 6258, 6257, 5353 and 5351, respectively mean = 19.25 ± 0.47; n = 4). The two repubertual females, goat #6258 and 6257 showed first overt estrus on 22nd and 30th November, at the age of 248 and 251 days, respectively. This observation is in agreement with the results of Simaraks (1978) and Fitzgerald and Butler (1982) who reported the age at first ovulation in ewe lambs as 221.2 ± 0.6, and 220.9 ± 5.9 days, respectively. Later, Chakraborty (1989) reported age at puberty in the Anglo-Nubian male goats as 226.8 ± 0.8 days. It is probable that the prepubertal goats in this study may have reached puberty earlier but were unable to start cycling due to environmental conditions not being optimum for them to have done so. This is supported by the observation that they reached puberty at the same time that their elder dams were resuming their seasonal ovarian cyclicity.

Hormone level changes

Mean ± S.E. levels of LH, FSH, progesterone and estradiol-17 in the four goats are illustrated in Fig.1. The day of anestrus in normalized to the day of the first behaviourial estrus of the breeding season. LH and FSH levels fluctuated

randomly throughout anestrus. However, a two-to-three fold increase in their mean values was observed five days before the preovulatory surge prior to the first ovulation. After the first ovulation, mean levels of FSH decreased and were similar to those observed during anestrus. LH mean levels decreased to baseline levels that were lower than prior to the first ovulation by the third day after the preovulatory surge. Prior to the first observed estrus, both gonadotropins were secreted in the same pattern as observed prior to the first ovulation; there were again elevations (from 0.5 to 1.8 and 27.3 to 61.7 ng/ml for LH and FSH, respectively) in both gonadotropins two days before their preovulatory surge. Similar results have been reported by Huffman et al., 1987 in ewe lambs.

Meanwhile, mean progesterone concentrations were low during anestrus but increased by about four-fold (from an average of 0.5 to 2.3 ng/ml) two days before the first ovulation (Day-23). They then decline to low values on the day of first ovulation. This correlates with the work of Ojeda et al., 1984; Foster and Ryan, 1979; Kinder et al., 1987; and Ryan et al., (1991) who observed a small transient rise in progesterone of 4 - 7 days duration before declining to very low levels before first silent ovulation in ewe lambs. Greyling et al., (1990) reported similar findings in Boer kids attaining puberty. During the interval between the first presumed ovulation and the first behaviourial estrus, progesterone concentration and pattern of secretion were characteristic of that observed during the normal cycle of a goat (Thorburn and Schneider, (1972), Baird, (1992).

The rise in mean progesterone concentration two days preceding first ovulation has been observed in cows before the first post-parturient ovulation (Robertson, 1972; Nkuuhe et al., 1978), during the transition from anestrus to breeding activity in ewes (Yuthasastrakosol, 1975; Walton et al., 1977; Wheeler et al., 1977) and in ewe lambs upon reaching puberty (Simaraks, 1978; Baird, 1992). In the ewe and most probably in the goat progesterone in derived almost exclusively from the corpus luteum. Its secretion, therefore, is a measure of the activity of this ovarian compartment. The increase in the release of LH five days before first ovulation is thought to induce luteinization of one or more follicles which then secrete the increased level of progesterone that is seen a few days preceding first presumed ovulation (Wheeler et al., 1977; Walton et al., 1977). This may come about either as a result of insufficient release of LH or from the inherent incapability of the follicle(s) to ovulate in response to the stimulus. This hypothesis has been cited and may be supported by the observation that ovarianfollicles can grow and regress within 5 to 7 days (Smeaton and Robertson, 1971).

Serum mean estradiol-17 concentrations varied during anestrus and were observed elevated a day before the LH/FSH preovnlatory surge of the first presumed ovulation. After that, they remained low and declined gradually to their lowest levels on Day-7 before the first estrus. Beginning on Day-6 before the first behavioral estrus mean estradiol - 17 levels rose progressively peaking two days before the LH/FSH surge. This finding is in agreement with earlier studies and supports the hypothesis that rising levels of estradiol act synergistically with declining levels of progesterone to precipitate increased tonic LH secretion and the events that lead to ovulation as has been observed in sheep (Ryan et al., 1991).

Conclusion

Puberty as measured by the manifestation of first pyschic estrus was reached at the age of 251 and 248 days by two prepubertal goats, respectively. From the progesterone profiles, it appeared evident that all the four animals resumed (or started) their breeding activity by exhibiting ovulations that were not accompanied by overt estrus. Profiles of LH, FSH, progesterone, and estradiol obtained in this study indicate that the onset of the annual breeding season in the adult and puberty in the young appear to share a common mechanism, which is a decrease in the inhibitory feedback response to estradiol on LH secretion which will stimulate a transient progesterone rise from recruited follicle(s).

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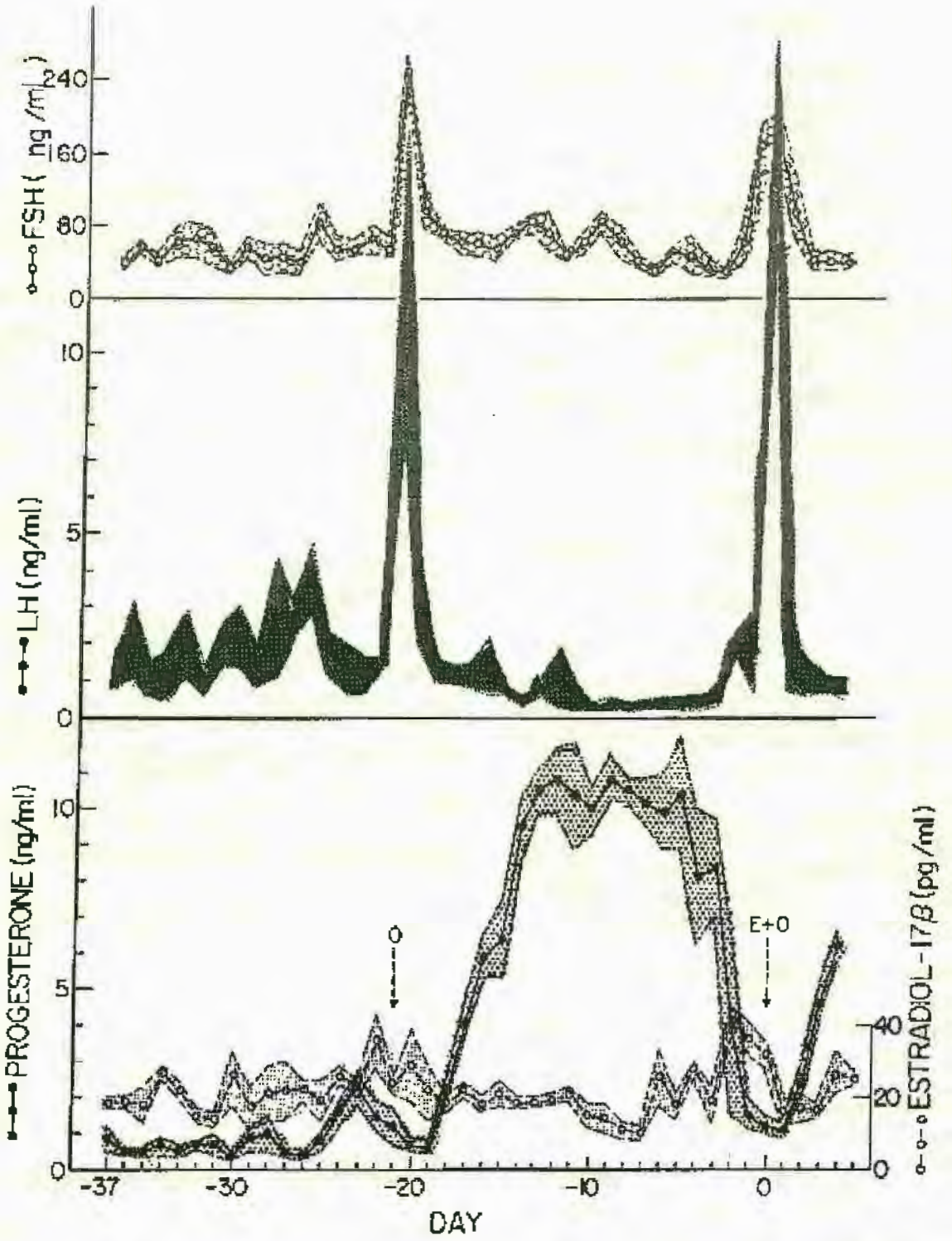

Figure 1. Composite hormone (mean ± S. E.) profiles for the 4 animals during transition from anestrus to breeding activity. (0 designates first presumed ovulation and E + 0 = first estrus and presumed ovulation).

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