

Effect of Blood Sampling and Administration of ACTH on Cortisol and Progesterone Levels in Ovariectomized Zebu Cows (*Bos indicus*)

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Bolaños, J.M., J.R. Molina and M. Forsberg: Effect of blood sampling and administration of ACTH on cortisol and progesterone levels in ovariectomized zebu cows (*Bos indicus*). Acta vet. scand. 1997, 38, 1-7. – Four zebu cows were bilaterally ovariectomized through lateral laparotomy. Three months after ovariectomy, blood samples were collected by jugular venipuncture daily for 5 consecutive days prior to a single injection of ACTH to establish baseline concentrations of cortisol and progesterone. Baseline concentrations of cortisol and progesterone were 31 ± 5 nmol/L and 0.3 ± 0.01 nmol/L, respectively. On the day of ACTH treatment the cows were allowed to rest for 2 h to reduce the stress of cannulation before the sampling period started. Blood samples were collected every 30 min from 2 h before until 2 h after the injection of $6 \mu\text{g}$ ACTH and hourly between 2-6 h after ACTH injection. A significant increase was observed in cortisol secretion from 90 min before until 120 min after ACTH injection. No significant increase was observed in progesterone secretion before ACTH injection. After ACTH injection progesterone was significantly elevated for 120 min. Four weeks after the ACTH treatment the cows were cannulated again and blood samples were collected following the same bleeding schedule used during the ACTH experiment. Instead of ACTH a saline injection was given via the catheter. A significant increase in cortisol concentration was recorded 90 min before saline injection. This increase was not accompanied by an elevation in progesterone concentration. No significant changes were observed in cortisol and progesterone levels after saline injection. When cortisol was added to a plasma pool having a progesterone concentration of 0.3 nmol/L and a cortisol concentration of 25.4 nmol/L and assayed for progesterone in 2 different assays no increase in progesterone concentration was observed. We conclude that the adrenal glands can be an extra-ovarian source of progesterone during stress in Zebu cows.

ACTH.

Introduction

Several stress conditions alter or depress reproductive performance in mammals (Levine & Urshin 1991, Rivier & Rivest 1991, Rivest &

Rivier 1995). In response to the stress stimuli glucocorticoids are secreted from the adrenal cortex mediated by CRF (corticotrophin-releasing factor) from hypothalamus and with subsequent ACTH release from the anterior pituitary gland.

Cortisol is the predominant glucocorticoid in

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bovine blood and has been used as a reliable endpoint to determine the response to stress and disease of the adrenal cortex (Venkateshu & Estergren 1970). Stoebel & Moberg (1982) reported that adrenal activity increases in cows submitted to stress with an increase in circulating cortisol levels.

Progesterone is known to be secreted by the adrenal glands of rats (Resko 1969, Fajer et al. 1971), ewes (Green & More 1977), cows (Gwasdauskas et al. 1972, Wagner et al. 1972), red deer (Jopson et al. 1990), white-tailed deer (Wesson et al. 1979, Plotka et al. 1983) and fallow deer (Asher et al. 1989). Watson & Munro (1984) showed that administration of increasing doses of ACTH to ovariectomized cows (*Bos taurus*) produced a dose-response curve of progesterone in peripheral blood. The authors concluded that progesterone was produced by the adrenal glands in response to low doses of ACTH, similar to natural stress, and was of sufficient magnitude to interfere with reproduction and influence fertility.

In the zebu cow, the level of stress reached when animals are submitted to intense management or experimental procedures is notably high. Furthermore, high ambient temperature has been reported to influence the length of the oestrus cycle and reduce the intensity of oestrus signs (Abilay et al. 1975, Hein & Allrich 1992). Lamothe-Zavaleta et al. (1991a,b) reported that cortisol secretion was elevated in zebu cows during the rainy season and that progesterone secretion in the beginning of the luteal phase was higher during the rainy season, with high ambient temperature, as compared to the dry season.

In a post-partum study performed in zebu cows in Costa Rica, unexpected fluctuations in progesterone after parturition were observed (Bolaños & Molina 1994) indicating that either progesterone or some other compound produced in response to the stress of handling and

blood sampling was measured in the progesterone assay.

The objective of the present study was to quantify the progesterone response to blood sampling and administration of synthetic ACTH in ovariectomized zebu cows, evaluating the adrenal glands as a possible secondary source of progesterone and the potential influence of acute stress on reproductive function in *Bos indicus* cattle.

Materials and Methods

Animals and experimental design

The study was carried out in the humid tropical area of Costa Rica in the region of San Carlos, Alajuela located at latitude 9° 22' N, 84° 33' W and 75 m above sea level. The average yearly rainfall is 3100 mm and relative humidity 82.5% with an annual mean temperature of 27.3°C.

Four zebu cows, aged 60 ± 0.9 (mean ± SEM) months and weighing 432 ± 26 kg, were bilaterally ovariectomized by lateral laparotomy. The cows were clinically healthy when selected for the experiment and were carefully monitored during the experimental period. The cows grazed on natural pastures with free access to water. No supplemental feed was offered during the experimental period.

Three months after ovariectomy blood samples were collected into heparinized Monoject tubes (Shewood Medicals, St. Louis, MO, USA) by jugular venipuncture daily for 5 consecutive days prior to the injection of ACTH to establish a baseline for cortisol and progesterone. Plasma was immediately separated by centrifugation and stored in plastic cryovials (Cryoware, Nalgene Company, Rochester MA, USA) at -20°C until assayed.

On the day of ACTH treatment animals were fitted with an indwelling jugular catheter without anaesthesia at 7:30 am. After catheterization the cows were allowed to rest for 2 h to re-

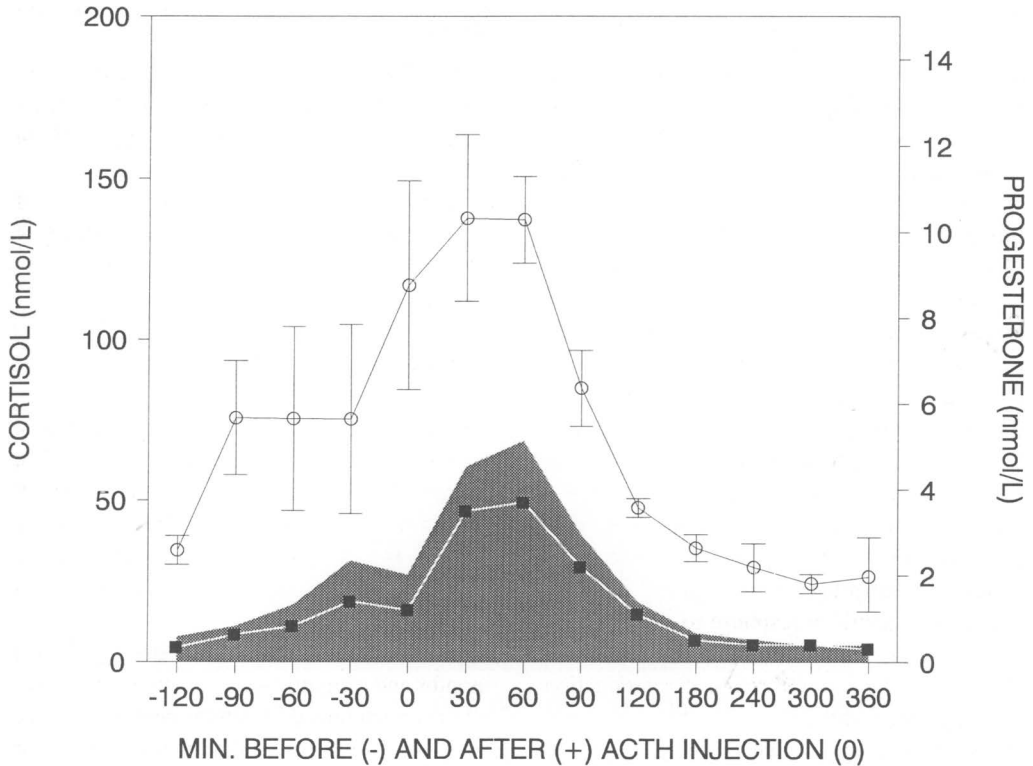


Figure 1. Temporal changes in plasma concentrations of cortisol (○) and progesterone (■) (mean ± SEM) before and after ACTH injection in 4 Zebu cows. The shaded area shows the maximal concentration of progesterone. The intravenous injection of ACTH was given directly after the blood sample at time 0.

duce the stress of cannulation, and then the sampling period started. Blood samples were collected every 30 min from 2 h before until 2 h after an intravenous injection of 6 µg ACTH (Synacthen Depot, CIBA-GEIGY, Basile, Switzerland; *Alam et al.* 1986, *Van der Kolk & Breukink* 1991) and hourly between 2-6 h after ACTH injection. The ACTH injection was given via the jugular catheter after the fifth blood sample.

Four weeks after the ACTH treatment the cows were cannulated again and blood samples were collected following the same bleeding schedule used during the ACTH experiment. Instead of

ACTH a saline injection was given via the catheter.

Hormone assays

Progesterone was determined by a Coat-A-Count DPC kit (Diagnostic Products Corporation, Los Angeles, CA, USA). Serial dilutions of bovine plasma with high concentrations of progesterone produced inhibition curves parallel to the standard curve. The sensitivity of the assay was 0.1 nmol/L. According to the manufacturer the antiserum shows no cross-reactivity with cortisol. The intra-assay coefficients of variation for 3 control samples (low, 2.3

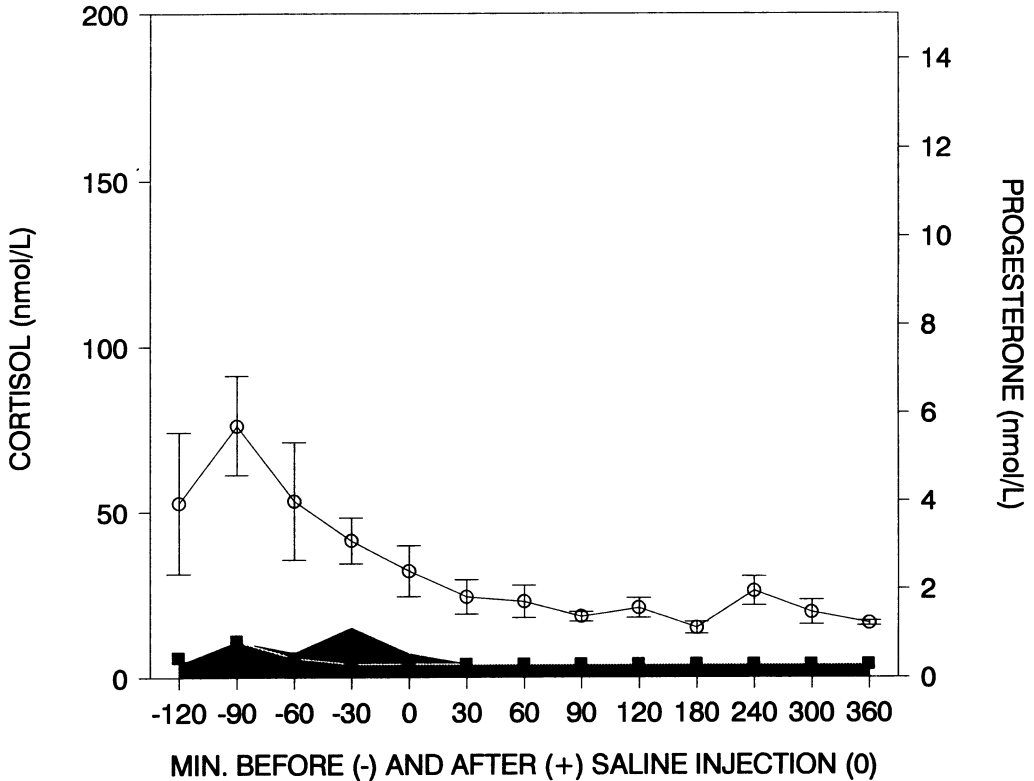


Figure 2. Temporal changes in plasma concentrations of cortisol (○) and progesterone (■) (mean ± SEM) before and after saline injection in 4 Zebu cows. The shaded area shows the maximal concentration of progesterone. The intravenous injection of saline was given directly after the blood sample at time 0.

nmol/L; medium 25.7 nmol/L and high 74.2 nmol/L) assayed in duplicates in 20 assays were 10.6%, 4.7% and 7.1%, respectively. The corresponding inter-assay coefficients of variation were 8.9%, 10.1% and 13.3%.

Cortisol was determined by a Coat-A-Count, solid phase radio immunoassay kit (Diagnostic Products Corporation, Los Angeles CA, USA). The detection limit of the assay was 5.5 nmol/L. According to the manufacturer the antiserum shows low cross-reactivity with progesterone (0.15%). Quality control samples containing endogenous cortisol were assayed in duplicates at the beginning and end of each assay. The

intra-assay variation was between 2.2% and 6.3%. The inter-assay coefficient of variation was between 3.8% and 5.2%.

To investigate the degree of cross-reactivity of cortisol with the Coat-A-Count progesterone antibody 0, 62.5, 125, 187.5, and 250 nmol/L of cortisol were added to a zebu plasma pool.

Statistical methods

Calibration curves, intra-assay and inter-assay coefficients of variation were calculated using procedures available in the Multicalc System (Wallac, Turku, Finland). The data were analysed by analysis of variance (Statistica, Stat-

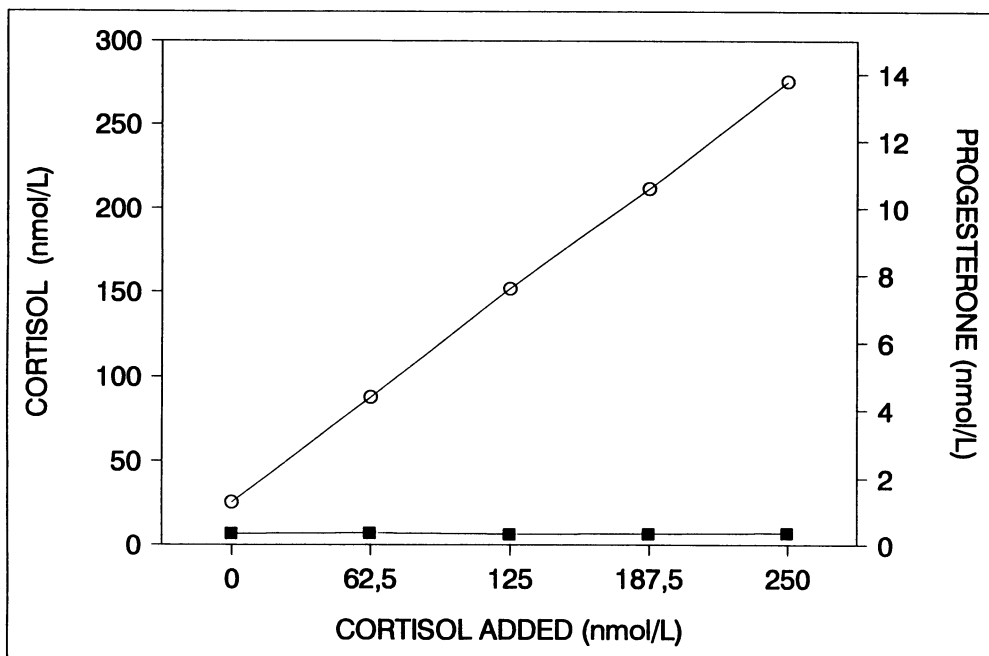


Figure 3. Concentrations of cortisol and progesterone measured in a pool of zebu plasma after addition of increasing concentrations of cortisol (62.5, 125, 187.5 and 250 nmol/L). The initial concentration in the pool was 0.3 nmol/L progesterone and 25.4 nmol/L cortisol.

Soft Inc., Tulsa, OK, U.S.A). Within-treatment group comparisons were made by the LSD-test. All statistical tests having a $p < 0.05$ were considered significant. Results are expressed as mean \pm SEM.

Results

Baseline concentrations of cortisol and progesterone were 31 ± 5 nmol/L and 0.3 ± 0.01 nmol/L, respectively. A significant increase was observed in cortisol secretion from 90 min before until 120 after ACTH injection. No significant increase was observed in progesterone secretion before ACTH injection. After ACTH injection progesterone was significantly elevated for 120 min (Fig. 1).

A significant increase in cortisol concentration was recorded 90 min before saline injection.

This increase was not accompanied by an elevation in progesterone concentration. No significant changes were observed in cortisol and progesterone levels after saline injection (Fig. 2). When cortisol was added (up to a 250 nmol/L) to a pool of zebu plasma having a progesterone concentration of 0.3 nmol/L and cortisol concentration of 25.4 nmol/L and assayed for progesterone, no increase in progesterone concentration was observed as shown in Fig. 3.

Discussion

It is evident from this study that the stress of blood sampling can produce an increase in cortisol levels in zebu cows. Similar results have been reported by *Alam & Dobson* (1986) and *Alam et al.* (1986) who observed that not only the insertion of the jugular catheter produced a

stress response in the cow (*Bos taurus*). Simple manipulation of the uterus, intramuscular or intravenous injections as well as blood sampling by venipuncture also produced increments in cortisol levels with return to baseline after 1 to 3 h.

After ACTH injection there was a concomitant increase in measurable progesterone, assayed with the Coat-A-Count procedure, correlating with the pattern of cortisol secretion. When facing these results we decided to repeat measurements of all samples in another progesterone assay (Amerlite, Kodak Clinical Diagnostics Ltd. UK) previously validated for bovine plasma (Duchens et al. 1995). The same progesterone pattern was reproduced, but mean levels were consistently lower ($r = 0.88$; Progesterone(Coat-A-Count) = $0.7 + 1.3 \times$ Progesterone (Amerlite)). However, cortisol itself is not cross-reacting with the progesterone antibodies. The manufacturers of the 2 kits report low or undetectable cross-reactivities to cortisol of their progesterone antibodies. We could also confirm this information since cortisol added to plasma samples did not produce changes in progesterone concentrations, indicating that either progesterone of extra-ovarian origin or some other steroid of structural similarity is being measured in both progesterone assays.

Our results show that stress is a factor that must be considered when reproduction of the zebu cow is studied. The adrenal glands may be an extra-ovarian source of progesterone. Such extra-ovarian secretion of progesterone could influence the interpretation of results in investigations of ovarian function in *Bos indicus* cattle.

Acknowledgements

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Sammandrag

Effekt av blodprovstagning och ACTH injektion på blodkoncentrationerna av cortisol och progesteron hos ovarieektomerade zebu kor (Bos indicus).

Tre månader efter att 4 zebu kor hade ovarieektomerats togs dagliga blodprover under 5 dagar för att fastställa basnivåerna i blod av cortisol och progesteron innan djuren gavs en injektion av ACTH. Basnivån för cortisol var 30 ± 5 nmol/L och för progesteron 0.3 ± 0.01 nmol/L. Samma dag som ACTH injektionen gjordes sattes en kateter in i jugularvenen och djuren lämnades i fred under 2 timmar innan blodprovstagningen påbörjades. Blodprov samlades var 30 minut från 2 timmar före till 2 timmar efter injektion med $6 \mu\text{g}$ ACTH och därefter varje timme 2-6 timmar efter injektionen. Kortisolnivån var signifikant förhöjd 90 minuter före till 120 minuter efter ACTH injektionen och progesteronnivån var signifikant förhöjd efter ACTH injektionen. Fyra veckor senare upprepades proceduren med den skillnaden att istället för ACTH injicerades en koksaltlösning. En signifikant ökning av kortisolnivån sågs 90 minuter innan koksalt injektionen. Denna ökning sammanföll inte med någon signifikant stegring i progesteronnivån. Inga signifikanta förändringar i blodkoncentrationerna av vare sig cortisol eller progesteron kunde ses efter koksaltinjektionen. När cortisol i stigande koncentrationer tillsattes plasma och proverna analyserades med 2 olika metoder ökade kortisolkoncentrationen medan motsvarande stegring i progesteronkoncentrationen uteblev. Experimentet styrker att progesteron inte enbart utsöndras från äggstockarna. I situationer av stress är förmodligen binjurarna en källa för progesteronproduktion.

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