Plasma Luteinizing Hormone and Progesterone Concentrations in Azawak Zebu Cows Submitted to Different Estrus Synchronization Protocols

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Keywords

Bos indicus – Azawak cow – Estrus synchronization – LH – Progesterone – Radioimmunoassay – Burkina Faso.

Summary

The percentage of estrus induction and plasma LH concentrations were examined in 15 Azawak zebu cows submitted to two different estrus synchronization protocols. In the first treatment (T1, n = 9), the cows received a norgestomet ear-implant for 10 days associated with estradiol valerate, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and pregnant mare serum gonadotropin (PMSG) injections. Treatment 2 (T2, n = 6) consisted in two $PGF_{2\alpha}$ injections 11 days apart, followed by the administration of PMSG two days after the second prostaglandin injection. Blood samples were collected every three hours during five days after implant removal (T1) or PMSG injection (T2) for LH measurements. The proportion of induced estrus was higher in T2, but the difference was not significant. In general, the elapsed time until the estrus onset tended to be shorter in norgestomet-treated animals (35.9 ± 3.9 h) than in prostaglandin-treated ones (49.5 ± 5.8 h). Seven out of nine norgestomet-treated cows, and only two out of the six treated with $PGF_{2\alpha}$, presented a peak of LH. The mean interval from the end of treatments to LH peak tended to be longer in $PGF_{2\alpha}$-treated females than in norgestomet-treated ones. One female presenting abnormally high LH concentrations after implant removal did not show a peak of LH during the observation period.

■ INTRODUCTION

Estrus synchronization is a valuable management tool that has been used to enhance reproductive efficiency (4). One of the advantages of estrus synchronization is that a large number of females can be bred over a short period. Synchronization also allows farmers to schedule calving so as to take advantage of feed supplies and to develop breeding technologies such as artificial insemination and embryo transfer. Although the merits of estrus synchronization are best realized in large animal breeding structures, this technique can be used to benefit smallholder agropastoral farmers (4, 14).

A limited amount of research data suggests that there are physiological and behavioral differences between *Bos indicus* (zebu) and *Bos taurus* (taurine) cattle which may influence responses to estrus synchronization treatments (4, 6). Some of these hypothetical differences include a reduced capacity for LH secretion in *Bos indicus* compared to *Bos taurus* breeds (28) and a greater sensitivity of *Bos indicus* cows to exogenous gonadotrophins (19). Additionally, the timing of LH surge and ovulation can occur earlier in relation with the onset of estrus in *Bos indicus* (24), while behavioral estrus appears to be shorter and less overt in *Bos indicus* (9, 15, 23). Lower peripheral concentrations of progesterone have also been reported in *Bos indicus* cattle (24, 30).

The study was carried out to investigate the behavioral and endocrine (LH, progesterone) changes following synchronization of estrus in Azawak zebu cows by use of norgestomet and cloprostenol based protocols.

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■ MATERIALS AND METHODS

The experiment took place from November 1999 to Mars 2000 at the Ouagadougou University Farm of Burkina Faso (12°35'N, 1°50'W). Mean annual precipitation was 748 mm/year. Mean monthly minimum and maximum temperatures ranged from 26 to 39°C in the dry season (November to May).

The Azawak cow is a lightly built breed originating from the Azawak Valley (northeast of Nigeria) and distributed over the sub-Sahelian zone of West Africa (3). The height at withers range from 120 to 130 cm. The average weight of adult animals is 200–250 kg for females and 250–300 kg for males. Although, the coat color is usually red or light fawn, it can also be white, brown or pied and black.

Fifty-two Azawak zebu females of mixed age (3 to 7 years old) and parity were palpated per rectum to determine reproductive soundness and status one month before the beginning of the study. The mean corporal weight was 208 ± 24.5 kg. The females were maintained under a semi-intensive herd management system, grazing typical vegetation (savanna) and having free access to water and mineral blocks.

Fifteen females diagnosed as non pregnant were randomly selected and allocated into two different estrus synchronization protocols. Cows in treatment 1 (T1, n = 9) received an ear subcutaneous implant containing 3 mg of norgestomet plus intramuscular injections of 3 mg of norgestomet and 5 mg of estradiol valerate (Crestar, Intervet International, The Netherlands) on day 0 of the synchronization program. At day 8 after implant insertion, the cows received 500 µg of cloprostenol sodium ($PGF_{2\alpha}$, Estrumate, Intervet). At the time of implant removal (day 10), all animals received 400 IU i.m. of pregnant mare serum gonadotropin (PMSG) injections (Folligon, Intervet).

Treatment 2 (n = 6) consisted of two deep intramuscular injections of 500 µg of $PGF_{2\alpha}$ (Estrumate, Intervet) eleven days apart. Forty-eight hours after the second $PGF_{2\alpha}$ injection, 400 IU i.m. of PMSG (Folligon, Intervet) were administered to each female.

Estrus detection was conducted by visual observation of mucus discharge at six-hour intervals during five days. The synchronized estrus response was defined as standing estrus within five days of implant removal (T1) or PMSG injection (T2). Mounting activities or females standing to be mounted by other females were also recorded.

Blood was obtained by jugular venipuncture into 7.5 ml S-Monovette potassium-EDTA tubes (Sarstedt, Germany). The samples were immediately centrifuged at 1500 *g* for 15 min to remove plasma. Plasma was aliquoted and then stored at –20°C until assayed for LH and progesterone.

For the LH assay, blood samples were collected every three hours after implant removal (T1) and the second $PGF_{2\alpha}$ injection (T2) until the 120th hour. For the progesterone assay, blood samples were taken at 5 to 10 day intervals till at least one month after estrus synchronization.

In order to compare the efficacy of both treatments, cows were systematically inseminated 48 and 72 h after implant removal (T1) or PMSG injection (T2) independently of estrus detection.

LH radioimmunoassay

LH concentrations were determined by a double antibody radioimmunoassay (RIA). The LH preparation used in the standard curve (0.2 to 25 ng/ml) and for iodination (34) was from ovine origin (Ovine LH-1-2; NIH, USA). Serum was from goat origin. The first antibody (L34) was raised in rabbits as described elsewhere (35) and used at a final dilution of 1:100,000. The double antibody precipitation system was composed of a mixture of sheep anti-rabbit immunoglobulin (0.83% v:v), normal rabbit serum (0.17% v:v), polyethylene glycol 6000 (20 mg/ml; Vel, Belgium), cellulose microcrystalline (0.05 mg/ml; Merck, Germany) and bovine serum albumin (BSA, 2 mg/ml; ICN Biochemicals, USA) diluted in Tris buffer (25 mM Tris, 10 mM $MgCl_2$ and 0.1 mg/ml neomycin sulfate; pH 7.5).

In summary, 0.1 ml of each sample and appropriate standard dilution were aliquoted into duplicate assay tubes and diluted with 0.2 and 0.1 ml of Tris-BSA buffer, respectively. To minimize nonspecific interference of plasma proteins, 0.1 ml of LH-free plasma was added to all standard tubes. Then, 0.1 ml of the diluted antiserum was added and the tubes were incubated overnight at room temperature. The following day, 0.1 ml of radiolabelled ^{125}I-LH (25,000 counts min^{-1}) was added to all tubes, which were then incubated for 4 h before adding the double antibody precipitating system (1.0 ml). After further 30 min incubation, 2.0 ml of Tris-BSA buffer were added to all except total count tubes. Bound and free LH were separated by centrifugation (1500 *g* for 20 min). The supernatants were discarded and the radioactivity of the pellet was determined using a multigamma counter (LKB Wallac 1261; Finland) with a counting efficiency of 75%.

Progesterone radioimmunoassay

The progesterone plasma concentrations were estimated by an indirect method, after an ether petroleum extraction initial step (25). The progesterone (Sigma, USA) was diluted in assay buffer (phosphate buffered 0.1 M containing 0.15 M NaCl and 1.0 g/ml gelatin; pH 7.0) and used as standard (0.15 to 20 ng/ml). Progesterone-11-hemisuccinate-2[^{125}I]-iodohistamine was prepared by the mixed anhydride method (20). Purification of the tracer was carried out by high-performance liquid chromatography on C-18 reverse phase. The first antibody (R43) was raised against progesterone-11-hemisuccinate-BSA and used at a final dilution of 1:15,000.

Briefly, volumes of 0.2 ml of zebu sera were extracted with 3.0 ml of petroleum ether 60-80° (Petroleum spirit; BDH, UK) by shaking for 2 min. After centrifugation (1500 *g* for 10 min), the aqueous phase was frozen in liquid nitrogen and the organic phase transferred to glass tubes. The solvent was then removed in an evaporator and the residue redissolved in 0.3 ml of phosphate assay buffer under moderate agitation (2 h at 37°C). Labeled progesterone (0.1 ml corresponding to 30,000 cpm) and first antiserum (0.1 ml) were added and an incubation was performed for 3 h at room temperature. Bound and free P4 were separated after addition of the double antibody precipitation system, as previously described for the LH assay. The intra- and inter-assay coefficients of variation of progesterone radioimmunoassay were 12.3 and 13.0%, respectively. Sensitivity was 0.2 ng of P4/ml.

Statistical analysis

The results were expressed as means (± SD). The effect of the estrus synchronization treatment on the different variables (estrus response, interval to estrus and LH concentrations) were compared using the chi-square test with the correction of Yates. Time for LH peak was defined as the elapsed time between the end of the estrus synchronization treatments and the maximum LH concentrations

Table I

Estrus induction and LH responses in Azawak zebu cows submitted to two distinct protocols of estrus synchronization

	Females exhibiting estrus	Time to estrus (h)	Time to LH peak (h)	Mean LH peak concentration (ng/ml)	LH baseline level (ng/ml)	Pregnant females
Norgestomet-treated females	8 (n = 9)	35.9 ± 3.9 (n = 8)	40.3 ± 4.5 (n = 7)	31.5 ± 16.2 (n = 7)	3.3 ± 1.3 (n = 7)	3
$PGF_{2\alpha}$-treated females	3 (n = 6)	49.5 ± 5.8 (n = 3)	54.0 ± 8.5 (n = 2)	22.4 ± 17.2 (n = 2)	3.5 ± 1.0 (n = 2)	1

LH: luteinizing hormone; $PGF_{2\alpha}$: prostaglandin $F_{2\alpha}$

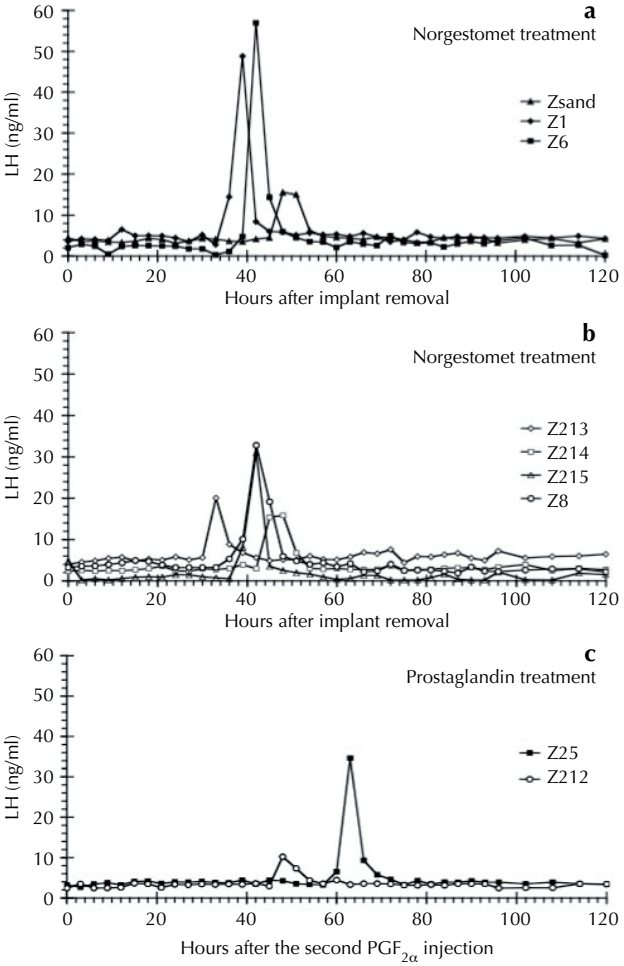

Figure 1: *Plasmatic LH concentrations in Azawak zebu cows treated with norgestomet ear-implant for 10 days associated with estradiol valerate, prostaglandin-$F_{2\alpha}$ and pregnant mare serum gonadotropin injections (1a, 1b) or with two $PGF_{2\alpha}$ injections 11 days apart (1c). Cows which became pregnant are shown with black-filled symbols.*

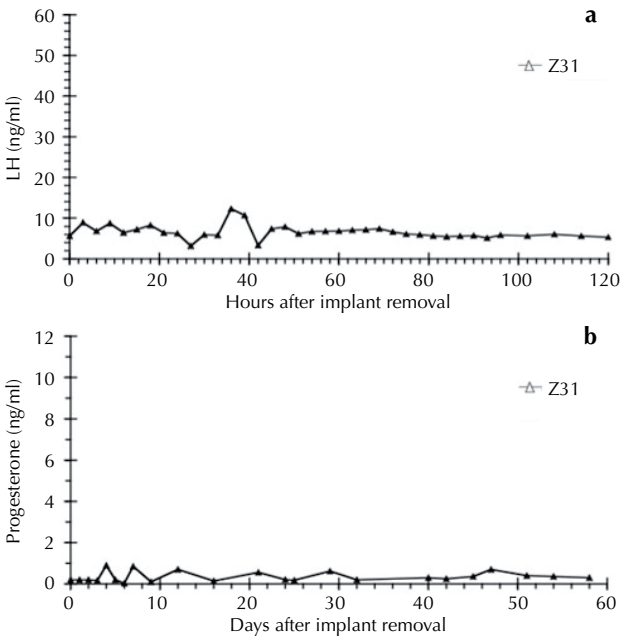

Figure 2: *Typical high LH concentrations in one Azawak zebu cow treated with a norgestomet ear-implant for 10 days associated with estradiol valerate, prostaglandin-$F_{2\alpha}$ and pregnant mare serum gonadotropin injections (2a). Low progesterone levels after estrus synchronization (2b).*

that occurred in cows with identifiable LH peak. Statistical tests having a value of $P < 0.05$ were considered significant.

■ RESULTS

Table I summarizes estrus induction, LH responses and pregnancy rates in Azawak zebu cows submitted to two different estrus synchronization treatments. The proportion of females exhibiting estrus within 120 h after the end of the treatment was not significantly affected by the treatment. In general, norgestomet-treated animals were the first to be in estrus (35.9 ± 3.9 h vs 49.5 ± 5.8 h), but this difference was not significant.

Seven out of nine norgestomet-treated cows presented an LH peak compared with 2 $PGF_{2\alpha}$-treated zebu females (Table I). The mean interval from the end of the synchronization protocol to the LH peak was slightly longer in $PGF_{2\alpha}$-treated zebu females.

LH concentrations

In the first treatment (Figure 1a and 1b), the highest LH peak concentrations ranged from 15.5 ng/ml (Zsand cow) to 57.0 ng/ml (Z6 cow), while in the second treatment (Figure 1c) the highest concentrations were 10.2 ng/ml (Z212) and 34.6 ng/ml (Z25). There was no significant effect of the synchronization protocol on the highest LH peak concentrations between treatments.

One norgestomet-treated cow (Z31) presented abnormally high basal LH concentrations after implant removal (Figure 2a). From the end of the treatment till the 120th hour after implant removal, mean LH concentrations (6.9 ng/ml) were approximately twice higher than those found in the other treated females.

Progesterone concentrations

Progesterone concentrations remained at basal levels (< 1.0 ng/ml) in the norgestomet-treated cow presenting an abnormally high basal LH concentration (Z31, Figure 2b), as well as in one cow (Z8) previously exhibiting an LH peak (Figure 3b).

Three cows from treatment 1 (Zsand, Z1 and Z6, Figure 3a) and one cow from treatment 2 (Z25, Figure 3c) presented progesterone concentrations, indicating the presence of a functional corpus luteum for a period longer than one to three months, suggesting successful pregnancies after estrus synchronization treatments. In the others (Z213, Z214 and Z215, Figure 3b, and Z212, Figure 3c), progesterone concentrations ranged from 1.91 (Z214) to 7.37 ng/ml (Z215) between days 6 and 9 after the end of the synchronization treatments, returning to basal levels between days 20 and 30 after the end of treatment.

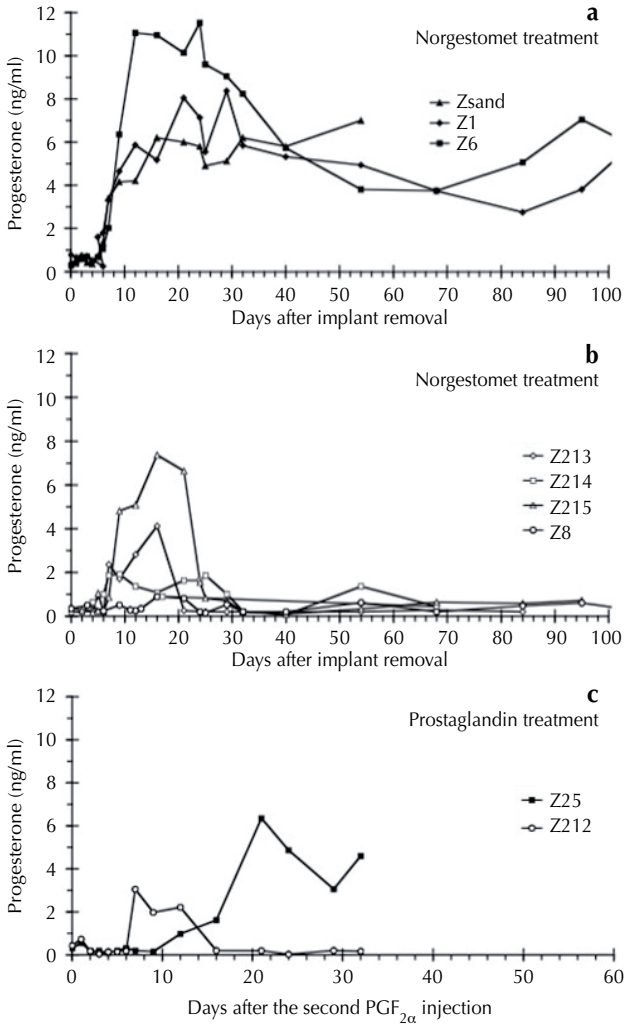

Figure 3: *Progesterone concentrations in pregnant and non-pregnant Azawak zebu cows treated with a norgestomet ear-implant for 10 days associated with estradiol valerate, prostaglandin-$F_{2\alpha}$ and pregnant mare serum gonadotropin injections (3a, 3b) or with two $PGF_{2\alpha}$ injections 11 days apart (3c). Pregnant cows are shown with black-filled symbols.*

■ DISCUSSION

There is abundant literature on hormonal profiles following estrus synchronization treatments in temperate breeds of cattle (21). However, there are few reports on the periovulatory hormone profiles in Azawaks that are administered different treatments for the synchronization of estrus (5, 18). In this study, the authors describe LH and progesterone plasmatic profiles in Azawaks submitted to norgestomet (T1) and cloprostenol (T2) estrus synchronization treatments. Estrus behavior and pregnancy rates are also reported.

The authors attempted to determine reproductive soundness and status of the Azawak females (rectal palpation one month before the beginning of estrus synchronization treatments), but the estrus response rates (8 of 9 animals in T1 and 3 of 6 in T2) were systematically lower than those obtained in Brahman, Bunaji and Indian zebu cows allotted into equivalent synchronization treatments (5, 16, 17, 36). On the other hand, results from T1 corroborated those obtained by Lokhande et al. (17) and Pinheiro et al. (23) who reported estrus responses varying by 64% in Indian zebu cows, and from 4 of 9 and 8 of 11 Nelore heifers and cows treated with subcutaneous ear-implant of norgestomet associated to estradiol valerate injection, respectively.

When compared with T1, Azawak females treated by two injections of cloprostenol eleven days apart had the lowest proportion of estrus behavior within 120 h after end of treatment. Low and variable estrus responses to prostaglandin treatments have been described in Nelore cows (13/28) and heifers (5/15) housed under tropical conditions (23). Also, poor estrus expression in Indubrazil zebu cows treated by prostaglandin (8/17 and 9/15 females) was described by Moreno et al. (18). Effectiveness of the use of prostaglandin and its analogs in estrus synchronization programs depends on the presence of a corpus luteum susceptible to luteolytic actions of these molecules (13, 37).

One explanation for the limited response of T2 in zebu cows is that some animals have been in anestrous before the cloprostenol treatment. It remains to be determined whether the absence of functional corpus luteum in the ovaries of some Azawak females is due to the poor nutritional quality of tropical pastures (27) or to other factors such as the hierarchical status or environmental conditions (5, 15).

The interval from the end of treatments and the beginning of estrus ranged from 35.9 ± 3.9 h to 49.5 ± 5.8 h in T1 and T2, respectively. As previously observed by several authors, the timing to exhibit estrus after the end of synchronization treatments is longer in prostaglandin-treated zebu cows than in progestogen-estradiol valerate treated ones (5, 23). Furthermore, intervals were consistently shorter in Azawak cows compared with Brahman and Nelore cows treated with both progestogen (41.9 and 57.7 h, respectively) and prostaglandin synchronization protocols (72 and 70 h, respectively) (5, 23).

One cow from each synchronization protocol was characterized as standing in estrus but did not present a peak of LH during the 120-h observation period. So, the proportion of females exhibiting estrus and presenting an LH peak remained relatively constant in both groups (7/8 in T1 and 2/3 in T2). Social interaction in large groups of cows treated to synchronize estrus contributes to modify the behavior of non-cyclic cows so that they can display signs of estrus (7, 8, 10, 33). However, a delayed time to LH peak or some errors in detection of estrus cannot be excluded to explain discordance between the number of cows exhibiting estrus and those presenting an LH peak (1).

Plasmatic LH concentrations increased markedly at estrus to reach 31.56 ± 16.23 ng/ml and 22.41 ± 17.25 ng/ml at 40.3 ± 4.5 h and 54.0 ± 8.5 h after the end of T1 and T2, respectively. In Brahman cattle, Cavalieri et al. (5) reported the highest LH concentrations 41.6 and 77.5 h after the end of progestogen- and prostaglandin-based treatments, respectively. However, peaks of LH concentrations found by these authors varied from 8 to 12 ng/ml. On the other hand, Rhodes and Randel (28) detected maximum LH concentrations varying from 20.2 to 113.2 ng/ml in Brahman and Holstein synchronized cows, respectively. Comparative data on cows under normal estrus cycles also show a smaller preovulatory LH surge in zebu compared with taurine breeds (24). Therefore, it remains unknown whether LH concentrations observed in Azawak females are mostly attributed to the radioimmunoassay technique used or to an important breed effect.

The baseline LH concentrations described in beef (0.79 ng/ml) and dairy cows (0.57 ng/ml) (12, 22) were four to six times lower than those observed in the present study (3.3 to 3.5 ng/ml). However, the present results are in the range of basal LH concentrations reported for Brahman cows (3.5 ng/ml) (28). Surprisingly, one progestogen-implant-treated Azawak cow (Z31) presented suprabasal LH concentrations all throughout blood sampling. To detect any errors within the procedure for this cow, the samples were assayed twice, confirming the results previously found. This cow also had low progesterone concentrations and, consequently, did not become pregnant after time-fixed artificial insemination (48 and 72 h after end of treatment). In another study, higher basal levels of endogenous LH were observed in cows who maintained body condition in the postpartum period (0.83 ng/ml), when compared to those who lost body condition (0.21 ng/ml) (29). However, it seems that there are no reports on such high (mean of 6.9 ng/ml) endogenous LH concentrations in cows in the scientific literature. The authors hypothesized that these suprabasal concentrations of LH could be associated to an absence of functional target receptors in the ovaries which leads to an abnormal synthesis of this hormone by the pituitary gland. On the other hand, recent studies have shown that basal LH release can be significantly increased after brain perfusion with galanin, a widespread expressed neuropeptide (2, 11). Further studies are necessary to verify the origin of suprabasal LH concentrations in some of the treated females.

Progesterone concentrations remained at basal levels (< 1.0 ng/ml) in one norgestomet-treated cow presenting abnormally high basal LH concentrations (Z31). Four treated cows (3 from T1 and 1 from T2) presented progesterone concentrations indicating the existence of a functional corpus luteum for a period longer than one to three months. Retrospectively, it appeared that cows that exhibited the higher LH peaks corresponded to those which became pregnant. The higher surges may result from a better feedback related to a better follicular growth and oocyte quality. In this context, the high LH surges are more probably a consequence than a primary cause of pregnancy. In non-pregnant cows, progesterone concentrations started to increase at days 6 to 9, returning to basal levels between days 20 and 30 after the end of treatment. The notion that LH is the primary luteotropic agent in cattle was first introduced by Simmons and Hansel as early as 1964 (31). Progesterone concentrations described in both pregnant and non-pregnant females were similar to those previously described in taurine (12) and zebu breeds (26, 32).

■ CONCLUSION

When compared to the prostaglandin estrus synchronization protocol, the progestogen-implant treatment led to a shorter time to cows standing in estrus and to LH peak. Plasma LH and progesterone concentrations in Azawak zebu cattle were similar to concentrations in other zebu breeds during the estrous cycle. In addition, the authors showed for the first time that suprabasal LH concentrations can be observed in cows failing to conceive after use of synchronization protocols.

Acknowledgments

This work was supported by grants from the International Foundation for Sciences of Sweden. Hormone assays were supported by grants from the Belgian Ministry of Agriculture. The authors gratefully acknowledge the Agence universitaire de la Francophonie for its financial support concerning researcher mobility and data analysis. Special thanks to Mrs R. Fares (ULg) for her secretarial assistance. Crestar, Estrumate and Folligon were gifts from Intervet International.

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Reçu le 16.05.2007, accepté le 12.06.2009

Résumé

Zongo M., Pitala W., Sawadogo L., Boly H., Melo De Sousa N., Sulon J., Beckers J.-F. Concentrations de l'hormone lutéinisante et de la progestérone chez des vaches zébus Azawak soumises à différents protocoles de synchronisation de l'œstrus

Quinze vaches zébus Azawak ont été soumises à deux protocoles de synchronisation de l'œstrus. Les concentrations plasmatiques de LH et le pourcentage des femelles ayant exhibé un comportement d'œstrus ont été répertoriés. Le premier traitement de synchronisation (T1, n = 9) a été effectué avec des implants sous-cutanés de norgestomet placés sous la peau de l'oreille pendant 10 jours. Le traitement a été complété par des injections de valérate d'œstradiol, de prostaglandine $F_{2\alpha}$ et de *pregnant mare serum gonadotropin* (PMSG). Le deuxième protocole (T2, n = 6) a consisté en deux injections de prostaglandine $F_{2\alpha}$ à 11 jours d'intervalle, suivies d'une injection de PMSG deux jours après la deuxième injection de prostaglandine. Des échantillons de sang ont été prélevés à trois heures d'intervalle pendant cinq jours après le retrait de l'implant (T1) ou l'injection de PMSG (T2) pour mesurer les taux de LH. Le taux d'œstrus induit a été plus élevé chez les vaches du groupe T2, mais cette différence n'a pas été significative. En général, le temps observé entre la fin du traitement et le début de l'œstrus a été plus court chez les femelles traitées au norgestomet (35,9 ± 3,9 h) que chez celles traitées à la prostaglandine (49,5 ± 5,8 h). Sept sur les neuf femelles traitées au norgestomet et seulement deux sur les six traitées à la prostaglandine ont présenté un pic de LH. L'intervalle moyen entre la fin des traitements et le pic de LH a eu tendance à être plus long chez les femelles traitées à la prostaglandine que chez celles traitées au norgestomet. Une femelle a présenté des concentrations de LH anormalement élevées après le retrait de l'implant. Toutefois, aucun pic de LH n'a été observé pendant la période de suivi.

Mots-clés : *Bos indicus* – Vache Azawak – Synchronisation de l'œstrus – LH – Progestérone – Technique radioimmunologique – Burkina Faso.

Resumen

Zongo M., Pitala W., Sawadogo L., Boly H., Melo De Sousa N., Sulon J., Beckers J.-F. Concentraciones de progesterona y de hormona plasmática luteinizante en vacas Cebú Azawak sometidas a diferentes protocoles de sincronización de estro

Se examinó el porcentaje de inducción de estro y las concentraciones de LH en plasma en 15 vacas Cebú Azawak sometidas a dos protocolos diferentes de sincronización de estro. En el primer tratamiento (T1, n = 9), las hembras Azawak recibieron un implante de oreja norgestomet durante 10 días, asociado con valerato de estradiol, prostaglandina $F_{2\alpha}$ ($PGF_{2\alpha}$) e inyecciones de gonadotropina sérica de yegua preñada (PMSG). El tratamiento 2 (T2, n = 6) consistió en dos inyecciones de $PGF_{2\alpha}$ a 11 días de intervalo, seguido por la administración de PMSG dos días después de la segunda inyección de prostaglandina. Las muestras de sangre se recolectaron para medir la LH cada tres horas y durante cinco días después de la eliminación del implante (T1) o de la inyección de PMSG (T2). La proporción de estros inducidos fue más elevada en el T2, sin embargo la diferencia no fue significativa. En general, el tiempo recorrido hasta la presentación del estro mostró una tendencia a ser más corto en los animales tratados con norgestomet (35,9 ± 3,9 h) que en los tratados con prostaglandinas (49,5 ± 5,8 h). Siete de cada 9 vacas tratadas con norgestomet presentaron un pico de LH comparable a 2 de cada 6 vacas tratadas con $PGF_{2\alpha}$. El intervalo promedio desde el fin del tratamiento hasta el pico de LH presentó una tendencia a ser más largo en las hembras tratadas con $PGF_{2\alpha}$ que en las tratadas con norgestromet. Una hembra, la cual presentó un nivel anormalmente alto de concentraciones de LH después de la eliminación del implante no mostró un pico de LH durante el periodo de observación.

Palabras clave: *Bos indicus* – Vaca Azawak – Sincronización del celo – LH – Progesterona – Técnica radioinmunologica – Burkina Faso.