

# Effects of equine chorionic gonadotropin (eCG) on the sexual behaviour and ovulatory characteristics of female african N'Dama cattle

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## ABSTRACT

This paper evaluated the effects of eCG on oestrus and ovulation in N'Dama heifers and cows synchronised by vaginal administration of progesterone (CIDR : Controlled Internal Drug Releasing Device) for seven days and a PGF<sub>2α</sub> injection two days after the end of treatment. The animals were randomly split into a non-treated control group (n=26) and four groups with increasing doses of eCG: 300 IU (n=25), 400 IU (n=24), 500 IU (n=23) and 600 IU (n=23), which were injected on the day of CIDR withdrawal. The animals were then followed (24 hours a day) for six days. Oestrus was defined by the time from first to last observed standing oestrus. Seven to ten days after the CIDR was withdrawn, the animals were slaughtered, and their ovaries were examined to identify and count the number of follicular and/or luteal structures. The onset of heat was  $48.6 \pm 5.3$  hours after eCG injection, and the average length of oestrus was  $9.9 \pm 2$  hours. The eCG dosage had no significant effect on these two parameters. In contrast, the observed rate of oestrus was significantly ( $p < 0.05$ ) higher in animals that had been treated (70.8 to 84.0%) in comparison with animals that had not been treated (50%). Similarly, the ovulation rate was significantly ( $p < 0.05$ ) higher in treated animals (88.0 to 100%) than in untreated animals (73.1%). The percentage of animals that presented multiple ovulations increased significantly ( $p < 0.001$ ) with the dosage of eCG administered (4.5% to 36.4%).

**Keywords:** N'Dama, equine Chorionic Gonadotropin, oestrus, ovulation

## RESUME

**Effets de la gonadotrophine chorionique équine (HCG) sur le comportement sexuel et ovulatoire des vaches de race N'Dama**

Cet article a évalué les effets de eCG sur l'oestrus et de l'ovulation de génisses et vaches N'Dama synchronisés par l'administration vaginale de progestérone (CIDR: Controlled Drug interne périphérique Releasing) pendant sept jours et une injection de PGF<sub>2α</sub> deux jours après la fin du traitement. Les animaux étaient répartis en un groupe témoin non traité (n = 26) et quatre groupes avec des doses croissantes d'eCG: 300 UI (n = 25), 400 UI (n = 24), 500 UI (n = 23) et 600 UI (n = 23) injectée le jour du retrait du CIDR. Les animaux ont été suivis (24 heures par jour) pendant six jours. La durée de l'oestrus était définie par le temps de la première à la dernière observation d'oestrus debout. Sept à dix jours après le retrait du CIDR, les animaux ont été abattus et leurs ovaires ont été examinés afin d'identifier et de compter le nombre de follicules et corps jaunes. L'apparition de la chaleur a été observée  $48,6 \pm 5,3$  heures après l'injection d'eCG, et la durée moyenne de l'oestrus était de  $9,9 \pm 2$  heures. La dose d'eCG administrée n'a pas eu d'effet significatif sur ces deux paramètres. En revanche, le taux observé d'oestrus a été significativement plus élevé ( $p < 0,05$ ) chez les animaux qui avaient été traités (70,8 à 84,0%) en comparaison avec les animaux non traités (50%). De même, le taux d'ovulation était significativement plus fort ( $p < 0,05$ ) chez les animaux traités (de 88,0 à 100%) que chez les non traités (73,1%). Le pourcentage d'animaux qui présentaient des ovulations multiples a augmenté de manière significative ( $p < 0,001$ ) avec la dose administrée d'eCG (4,5% à 36,4%).

**Mots-clés:** N'Drama, gonadotrophine chorionique équine, synchronisation, oestrus, ovulation

## Introduction

Given its rustic nature, its butchery quality and its trypanotolerance, the N'Dama breed represents a significant potential for developing African cattle breeding [6]. To date, its zootechnical performance and reproductive characteristics have been the subject of relatively little work. The use of selection programmes and artificial insemination are highly significant challenges for developing and improving this breed. Given the conditions under which these cattle are bred and hence the difficulty of inseminating animals when heat is observed, synchronised hormonal protocols have to be applied to allow systematic inseminations. Many different timed artificial insemination (TAI) hormonal protocols have been used in dairy and beef cattle under tropical conditions [2]. Some are based on the use of PGF associated or not to GnRH. The others involve the administration of progestagens for five to nine days, followed by an injection

of PGF<sub>2α</sub> and equine chorionic gonadotropin (eCG). Such protocols have been shown to allow systematic insemination [17, 20]. Exogenous progesterone suppresses LH release, and induces the appearance of a new wave of follicular growth four to five days later [12]. PGF<sub>2α</sub> is injected one or two days before the progesterone-based treatment is stopped or when it is stopped. This treatment aims to induce luteolysis in any corpora lutea (CL) present in cycled females. Administering eCG (equine Chorionic Gonadotropin) when the PRID is removed improves follicular growth and the ovulatory response. Stimulating the follicular growth and estrogens synthesis, eCG can improve the ovulatory response to a synchronization protocol without adverse effect following subsequent treatment [2]. The dosage of eCG should, nevertheless, be adjusted to the cycled or anoestrus status of the animal, so as not to induce superovulation, which is likely to lead to multiple pregnancies. The dosage and effects of eCG in female N'Dama have been the subject of relatively

little study. The authors have used doses of between 400 and 600 IU and have done so in animals with larger builds than N'Dama [14]. This study compares the effects of different doses of eCG within synchronisation protocols in N'Dama females on oestrus and ovulation. The onset, duration and manifestation of oestrus together with the ovulation rate and number of ovulations were evaluated.

## Material and methods

The study was conducted in Gabon (latitude: 2°15 North, 4° South; longitude: 8°30 West, 14°30 East) at the Nyanga Ranch between March and August 2013. The climate is equatorial, with annual average temperatures, relative hygrometry, and pluviometry of 24°C, 80% and 2,000 mm, respectively.

N'Dama heifers (n=64, 4.5 ± 0.7 years, 225.4 ± 18.6 kg and BCS = 2.5 ± 0.5) and primiparous females (n=57, 4.2 ± 0.5 years, 216.5 ± 20.7kg, and BCS= 2.4 ± 0.4) had already been treated three months before against trypanosomes by dipping in diminazene (Veriben<sup>®</sup>, 2,36g, Ceva Santé animale, Libourne, France) and isometamidium (Verigium<sup>®</sup>125mg, Ceva santé animale, Libourne, France) , against internal parasitoses through IM administration of levamisole (Quadrosol<sup>®</sup>, 10 %, Solartechnik-Schwabing, Munich, Germany) or ivermectine (IVOMEC D<sup>®</sup> 1%, Merial, Lyon, France), and against external parasitoses using flumethrin pour-on (Bayticol<sup>®</sup>, 1%, Bayer Animal Health, Loos, New Zealand). They were also vaccinated (Peri vaccine T1/SR) against Contagious Bovine Pleuropneumonia (CBPP). The animals grazed (one cow /4ha) on wild shrub land largely consisting of grasses, such as *Hyparrhenia Diplandra*. The animals received a bran supplement (3-5 kg/animal) and had unrestricted access to water and mineral salts.

The animals were weighed, and their body condition scores (BCS) were noted, taking into account the level of sub-cutaneous fatty deposits at the base of the tail on a scale from 1-5; 1=thin and 5=fat [1]. Genital tract and ovaries were examined through transrectal palpation and vaginoscopy at the insertion of intravaginal progesterone device (CIDR<sup>®</sup>, 1.38 g progesterone, Zoetis, Louvain-la-Neuve, Belgium). The presence or absence of a corpus luteum (CL) was determined at this point by transrectal ultrasonography (KX 5200 V ultrasound machine, Xuzhou Kaixin Electronic Instrument Company Ltd, 6.5 MHz linear probe). CIDR<sup>®</sup> was inserted for seven days [15] and PGF<sub>2α</sub> (Estrumate<sup>®</sup> 500 µg cloprostenol, Intervet, Brussels Belgium) was administered intramuscularly into each animal two days before removing the CIDR<sup>®</sup>.

Upon removal of the CIDR<sup>®</sup> day zero (D<sub>0</sub>), the animals were split into five experimental groups randomly when the animals passed through the cattle chute. In Group 0, 1 ml of physiological saline (Sodium chloride 0.9%: FRESINIUS KABI, Sevres, France) was injected intramuscularly (control group, n=26). eCG (Folligon<sup>®</sup> Intervet) was administered IM

to the animals in groups 1 (300 IU, n=25), 2 (400 IU, n=24), 3 (500 IU, n=23) and 4 (600 IU, n=23). A vaginoscopy was conducted on the day that the CIDR was removed to identify the animal's degree of tolerance. The mucus was examined and noted on a scale of 1 to 5 (1=absence of mucus, 5=abundant brown to red mucus). A value of less than 3 indicated a good tolerance of CIDR<sup>®</sup> [5].

After D<sub>0</sub>, the animals were permanently (ou continuously) observed (24 hours a day) for seven days to identify the onset and duration of oestrus. Onset of heat was defined as the period of time between the end of treatment and the start of oestrus. 'Female on oestrus' referred to all females that presented passive mount behaviour. Duration of oestrus' was defined as the time between the first and last acceptance of a passive mount.

All of the animals were slaughtered between D<sub>7</sub> and D<sub>10</sub>, and their ovaries were collected to identify the number and nature of structures present. Ovulation was confirmed on the basis of identifying a corpus luteum (CL) in the ovaries after the animal had been slaughtered. 'Anovulatory oestrus' referred to oestrus that was detected but was not followed by ovulation (no CL). 'Silent oestrus' referred to oestrus that was not detected but was followed by ovulation (at least one CL was present). The number of ovulations was determined by the number of CL in the ovaries.

The results are given as averages plus or minus the standard deviation (M ± SD). Data were processed using a general linear model and logistic regression. The fixed effects of the independent variables (whether eCG was injected, parity, cyclical status of the animal) and covariables, dose administered (linear and quadratic effect), age, weight, and BCS of the animals upon the onset and duration of heat, were studied using two analyses of variance (ANOVA). The use of four logistic regressions enabled the effects of these same variables (treatment, number of lactations, cyclicity) and covariables (dose, age, weight, BC) to be analysed in terms of their effects on the rate of inducing normal heat, silent heat, ovulation and multiple ovulation. All calculations were carried out using the SAS software (version 9.1).

## Results

The average age, weight and BCS of the animals were 4.4 ± 0.6 years, 221.2 ± 20 kg and 2.4 ± 0.4, respectively. No significant differences were observed among groups at inclusion (table I). The retention rate of the CIDR<sup>®</sup> device was 100%. Tolerance to the CIDR<sup>®</sup> was 77.7%, without any significant difference among the different groups.

In 86.4% of cases, the beginning of oestrus appeared between 6am and 6pm. The average percentage of cows observed in oestrus was 72.7%. This percentage was significantly ( $p < 0.05$ ) higher in animals treated using eCG. Moreover, this percentage correlated positively with the dose of eCG used (Table II).

After CIDR removal, the onset of oestrus occurred over a period of 41.5 hours and 73.9 hours, with an average of  $48.6 \pm 5.3$  hours. The delay between removal and oestrus onset was slightly higher in the absence of an eCG injection. This difference was, however, not significant (Table II).

The average length of oestrus was  $9.9 \pm 2$  hours, with values ranging from 5.5 hours to 13.5 hours. No significant difference was observed between animals in the control group and animals treated with eCG (Table II). The percentage of silent oestrus was, on average, 28.4%. This was significantly higher in the control animals. Similarly, a significant difference was observed among the experimental groups ( $p < 0.05$ ) (Table II). The average percentage of anovulatory oestrus was 4.5%. No significant differences were observed among the different groups of animals. Moreover, anovulatory heat was only observed in primiparous cows.

The total average percentage of cows that had ovulated was 90.1%. This was significantly higher ( $p < 0.05$ ) in heifers (95.3%) than in primiparous cows (84.2%). Moreover, this percentage appeared to be significantly higher in animals

that had been treated with eCG (88 to 100%) compared to control animals (73.1%) ( $p < 0.05$ ). In addition, this percentage increased significantly with the dose of eCG injected ( $p < 0.05$ ).

The average number of ovulations reported in cows that had ovulated ( $n=109$ ) was  $1.3 \pm 1.0$ . The numbers respectively were  $1.0 \pm 0$ ;  $1.0 \pm 0.2$ ;  $1.1 \pm 0.3$ ;  $1.4 \pm 0.7$  and  $1.3 \pm 0.5$ , for the control group and the groups treated with 300 IU, 400 IU, 500 IU and 600 IU. The average percentage of animals presenting several ovulations was 18.3%, and this was only observed in animals treated using eCG. In addition, we observed a highly significant increase ( $p < 0.001$ ) in this percentage for the doses of eCG between 300 and 500 IU, and no significant difference was observed between the doses of 500 and 600 IU.

## Discussion

The total average ovulation rate was 90.1%. Injection of eCG led to a significant increase in the ovulation rate. This percentage is comparable to that observed after treating dairy

Traits	Controls	300 IU	400 IU	500 IU	600 IU	RMSE	P-value
Numbers	26	25	24	23	23	NA	0.990
Age (years)	4.6	4.4	4.5	4.4	4.2	0,6	0.160
Weight (kg)	224.8	225	217.9	220.8	211.2	19,5	0.364
BCS	2.4	2.5	2.3	2.5	2.4	NA	0.730
- % heifers	65.4	52.0	45.9	60.9	39.1	NA	0.272
-% cycled animals	80.8	92.0	91.6	91.3	95.7	NA	0.187

IU = International Unit of equine chorionic gonadotropin. RMSE= Root Mean Square Error. BCS = Body Condition Score. NA = No Applicable.

TABLE I: Comparison of the characteristics of the different experimental groups. N'Dama females were treated with different doses of equine Chorionic Gonadotropin (eCG) after oestrus induction treatment.

Traits	Controls	300 IU	400 UI	500 UI	600 UI	RMSE	P-value
Numbers	26	25	24	23	23	NA	0.990
Onset of oestrus (h after CIDR removal)	52.0	47.3	48.4	48.7	47.8	5.2	0.241
Length of oestrus (h)	10.7	10.1	10.4	9.5	9.2	1.9	0.2821
Oestrus rate <sup>1</sup>	50.0 <sup>a</sup>	84.0 <sup>b</sup>	70.8 <sup>c</sup>	78.3 <sup>d</sup>	82.6 <sup>e</sup>	NA	0.0267
Silent oestrus rate <sup>2</sup>	53.8 <sup>a</sup>	9.5 <sup>b</sup>	41.2 <sup>c</sup>	27.8 <sup>d</sup>	21.0 <sup>e</sup>	NA	0.0137
Anovulatory oestrus rate <sup>3</sup>	7.7	4.8	5.9	5.6	0.0	NA	0.8586
Ovulation rate <sup>4</sup>	73.1 <sup>a</sup>	88.0 <sup>b</sup>	95.8 <sup>d</sup>	95.6 <sup>d</sup>	100.0 <sup>e</sup>	NA	0.0229
Multiple ovulations rate <sup>5</sup>	0.0 <sup>a</sup>	4.5 <sup>b</sup>	13.0 <sup>c</sup>	36.4 <sup>d</sup>	34.8 <sup>d</sup>	NA	0.0022

<sup>abcde</sup> (in each column) = significantly different numbers ( $P < 0.05$ ). RMSE = Root Mean Square Error. NA = Not applicable. \*\*\* = very significantly different numbers ( $P < 0.001$ ).

<sup>1</sup>Ratio between number of animals seen in oestrus and the number of treated animals. <sup>2</sup>Ratio between number of ovulating animals but not seen in oestrus and the number of animals seen in oestrus. <sup>3</sup>Ratio between number of not ovulating animals but seen in oestrus and the number of animals seen in oestrus <sup>4</sup>Ratio between number of ovulating animals and the number of animals of the group. <sup>5</sup>Ratio between animals with more than 2 ovulations and number of animals with at least one ovulation.

TABLE II: Effects of various doses of eCG on oestrus and ovulation characteristics in N'Dama females ( $n = 121$ ).

cows using a CIDR\* (79.2%) [26] or Nelore heifers treated using norgestomet (94.4%), [24] with an injection of 400 IU of eCG administered at the end of treatment. Because of its double FSH and LH activity, [3] eCG increases the pre-ovulatory peak of LH [25] and hence the percentage of ovulation observed [13,23].

The average number of ovulations was  $1.2 \pm 0.5$ . In 18,3% of case we have observed more than one ovulation, this percentage increasing with the dose of eCG. Similar results have been observed after use of 400 IU in dairy heifers ( $1.5 \pm 0.1$ ) [11] or 600 IU in beef heifers ( $4.0 \pm 1.1$ ) [10]. Similarly, in dairy heifers, a CIDR treatment followed by the injection of 400 IU of eCG and PGF<sub>2 $\alpha$</sub>  at the end of the treatment was accompanied by 12.5% multiple ovulations [26].

The average time between CIDR\* removal and oestrus was 48.6 hours. This value is comparable to that reported norgestomet intraauricular treatment of N'Dama females, which resulted in oestrus within 9 and 63 hours (36 hours on average), [7] after CIDR treatment in cows and beef heifers, this delay ranged from  $47.0 \pm 5.0$  hours to  $49.3 \pm 7.0$  hours [18]. In terms of the animals treated, 71.9 % were observed to come into heat within 72 hours of the end of treatment [18]. This rate was lower than that observed after treating N'Dama cows (females observed in heat reported against the total number of females treated) using a vaginal coil (80%) [28] or norgestomet (97.8%) [7]. It was also lower than that of heifers and dairy cows treated using a CIDR\* (80%) [15].

The average length of oestrus observed in this study was  $9.9 \pm 2$  hours. It was not influenced by eCG, regardless of the dose used. This duration was  $11.8 \pm 4.4$  hours for primiparous and  $10.8 \pm 3.8$  hours for multiparous Holstein cows [21]. In female N'Dama treated using norgestomet, the duration of heat was 11.1 hours [7]. Observation of heat using a teaser bull confirmed that oestrus lasted between 8 and 9 hours [19]. Quantified using an electronic mount detector, the duration of oestrus was between 2.6 and 26.2 hours (average 14 hours) in cross-bred heifers (Angus x Hereford x Brahman) treated for 14 days using melengestrol acetate [27]. In dairy cows, the average length of oestrus was  $7.1 \pm 5.4$  hours; 8.6 hours and  $9.6 \pm 6.9$  hours, respectively [9, 29, 30].

One female out of four (28.4%) was not observed to come into heat, despite the continuous observation methods used. This percentage was significantly higher in animals that were not treated with eCG (53.8% vs. 9.5 to 41.2%). N'Dama oestrus is known to be misleading [7, 19]. The injection of eCG was accompanied by an increase in the percentage of oestrus detected. Oestradiol 17 $\beta$  is the hormone responsible for oestrus behaviours [4, 8], with passive mounting activity being positively correlated with increased concentrations [16, 21, 22]. It appears that the injection of eCG is accompanied by greater follicular growth, and hence an increase in the production of oestradiol 17 $\beta$  [10,25,], encouraging better expression of heat.

## Conclusion

Attending the very high retention rate (100 %) , the use of a CIDR associated with eCG and PGF<sub>2 $\alpha$</sub>  can be recommended to synchronize oestrus in N'Dama cattle. Such treatment induces a good oestrus rate (71,6%) and a very high ovulation rate (90,1%). Due to this difference, a timed artificial insemination would be recommended.

## Acknowledgements

The authors thank the Programme for Institutional Support and Human Resources Development (PAI-DRH) in Gabon for funding the work, the Société d'Investissement pour l'Agriculture Tropicale (Siat-Gabon) for the framework and the University of Liège (ULg).

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