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REGULAR ARTICLES

Preovulatory follicle diameter, growth rate and time of ovulation during induced oestrus using a CIDR® in trypanotolerant female *Bos taurus* N'Dama cattle

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Abstract The aim of this study was to assess the dose (300 to 600 IU) effects of equine chorionic gonadotropin (eCG) on the preovulatory follicle diameter, growth rate and time of ovulation characterized by echography. The eCG was injected at the end (D_0) of the 7-day treatment with a controlled internal device release (CIDR®) and a $PGF_{2\alpha}$ being injected 2 days before the removal of the CIDR® (d_{-2}). The 120 N'Dama female were distributed into five experimental groups. The control group (n=26) was treated with physiological saline at the removal of the CIDR®, while the animals in the four treated groups received, respectively, 300 IU (n=25), 400 IU (n=24), 500 IU (n=22) and 600 IU (n=23) of eCG. The diameter of the preovulatory follicle was significantly higher (P<0.05) in the animals treated with 300 IU (10.1±1.4 mm) than in untreated animals (9.3±1.2 mm). Follicle growth rate was significantly (P<0.05) higher in treated animals (1.0±0.4 mm/day) than in the control group (0.9±0.4 mm/day). The average interval between the time of eCG injection and ovulation was similar in the non-treated (83.7±14.4 h) and treated animals (79.7±11.9). Treated animals showed a significant increase in the percentage of ovulation (94.7 % compared to 73.1 %) (P<0.01). Use of eCG contributed towards synchronising the time of ovulation between 72 to 96 h, which would facilitate the use of systematic insemination.

Keywords N'Dama cow · Follicular growth · Ovulation · CIDR · eCG

Introduction

Several studies showed that the diameter of the dominant follicle is one indicator of its ability to ovulate. In cattle, the dominant follicle ovulates when its diameter reaches between 15 and 20 mm (Lussier et al. 1987) or between 10 and 20 mm (Monniaux et al. 2009). The dominant follicle is capable of ovulating when its diameter is close to 10 mm (Ginther et al. 1996; Sartori et al. 2001). In heifers and beef cattle, the success of artificial insemination depends on gestation rates, which are themselves significantly influenced by the size of the follicle which has ovulated (Perry et al. 2005, 2007; Meneghetti et al. 2009). Maximising the size of the preovulatory follicle and the integrity of the ovocyte is therefore an important objective of reproductive hormonal management programmes which use systematic insemination (Wiltbank and Pursley 2014). In Senepol cattle (*Bos taurus*) living in tropical regions, which are closely related to the N'Dama (Williams et al. 1988), the diameter of the preovulatory follicle is 13.6±0.4 mm (Alvarez et al. 2000). To our knowledge, no study has been conducted to assess the size of the preovulatory follicle in N'Dama cattle. Moreover, the characteristics of oestrus in this breed make the rational use of artificial insemination difficult (Diop et al. 1998). Administering 300 IU of equine chorionic gonadotropin (eCG) and increasing the dose to between 400 and 600 IU, at the end of treatment with a CIDR®, increase the expression of oestrus and ovulation rates in females of this breed (Okouyi et al. 2014). In addition, hormone treatment protocols facilitating the use of systematic insemination remove the constraints of having to detect heat (Hanzen and Boudry 2003). To optimise the results of

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fertilisation and to improve gestation rates among N'Dama cattle, it is important to consider identifying the best time for systematic insemination in relation to ovulation. Ovulation takes place 26.7±5.4 h after the start of heat in dairy breeds (Walker et al. 1996). The interval between stopping progesterone-based treatment and ovulation varies between 69.6±2.4 h to 73.1±1 h in the Zebu Nelore (Sà Filho et al. 2010a) and from 65.4±2.2 to 66.0±3.3 h in other dairy breeds (Uslenghi et al. 2014). Few data are available in the literature on this interval in N'Dama cattle. This study was conducted to assess the diameter of the preovulatory follicle, the time of ovulation and the effects of 300 IU of eCG (and of increasing this to between 400 and 600 IU) after treatment with a controlled internal device release (CIDR®) on these parameters in female N'Dama cattle.

Materials and methods

The experiments were conducted in Gabon in the Nyanga ranch (Latitude: 2°15 North, 4° South; longitude: 8°30 West, 14°30 East) during the months of March to August 2013. The area in which the study took place has an equatorial climate which is hot (25–30 °C) and humid (80–85 % humidity) with high rainfall (1800–2000 mm). Vegetation consists of savannah shrub lands, interspersed with a few gallery forests which play host to the tsetse fly, the biological host and vector of trypanosomes, the agents responsible for African animal trypanosomiasis. Cattle feed on natural pastures consisting largely of *Hyparrhenia* sp. grasses. The load capacity is one tropical livestock unit for four hectares (1 TLU/4 ha). In addition, the animals regularly receive wheat bran (3–5 kg/animal/day). They have free access to water and mineral salts. They are regularly treated for parasites (levamisole/ivermectin) and are given preventative treatment against trypanosomiases (isometamidium chloride hydrochloride). They are vaccinated against pasteurellosis and contagious bovine pleuropneumonia (CBPP). The experimental group consisted of 63 heifers and 57 cows from the N'Dama breed. Each of them underwent a manual transrectal examination and ultrasound scan (KAIXIN® KX 5200V scan, Xuzhou Kaixin Electronic Instrument Company Ltd. using a linear sensor with a frequency of 6.5 MHz). They were weighed and their condition assessed on a scale of 1 to 5 (1=thin; 5=obese) using the (Ayres et al. 2009) method adapted to the N'Dama by assessing subcutaneous fatty deposits at the base of the tail.

After disinfecting the vulva region using a 5 % Ecutan® solution (chlorhexidine digluconate 50 mg/ml), a CIDR® (1.38 g of progesterone, Zoetis Belgium) was inserted for 7 days (Lucy et al. 2001) $PGF_{2\alpha}$ (2 ml of Estrumate®, 250 μg/ml of cloprostenol, Intervet Belgium) was administered IM to each animal 2 days before removing the CIDR®. After CIDR® removal (day 0), animals were split into five experimental groups. Animals of control group received an IM injection of 1 ml of physiological saline (sodium chloride 0.9 %: FRESENIUS KABI France) (control group, group 0: *n*=26). IM administration of 300, 400, 500 and 600 IU of eCG (Folligon®, 1000 IU/5 ml, Intervet Belgium) was respectively done to the animals in groups 1 (*n*=25), 2 (*n*=24), 3 (*n*=22) and 4 (*n*=23). These injections were done randomly as and when the animals passed through the pen. Each ovary was scanned to measure the diameter of follicles present on the day CIDR® withdrawal (day 0) and 2, 3, 4 and 5 days later (Fig. 1).

Ovulation was indirectly identified by the disappearance or sudden reduction between two consecutive measurements of the diameter of the dominant follicle (Alvarez et al. 2000; Castilho et al. 2007). The rate of growth of this dominant follicle (mm/day) was defined in relation to the difference between the follicle diameter observed prior to ovulation (day_{ov}) and that observed after removal of the CIDR® (day 0), divided by the number of days between removal and ovulation (Sà Filho et al. 2010b).

Time of ovulation

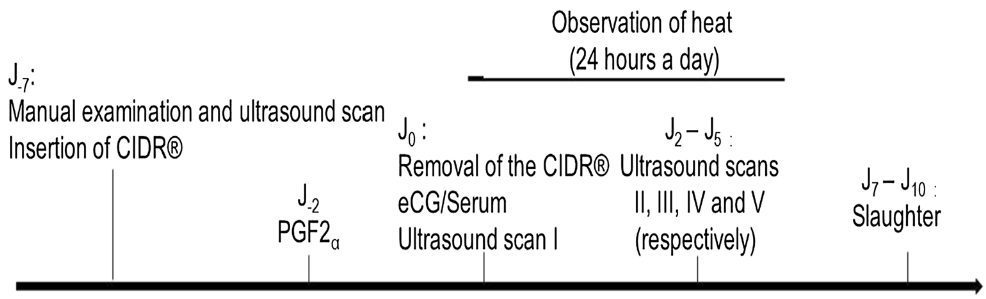
The animals were constantly observed (24 h a day) between the day of CIDR® withdrawal and their slaughter, to detect oestrus whose duration was determined by the time between the first and last standing heat.

All the animals were slaughtered (programed slaughter) 7 to 10 days after removal of the CIDR®. Ovulation was confirmed by identifying the corpus luteum on the ovary containing the identified dominant follicle.

Statistical analyses

The results were expressed as an average plus or minus standard deviation (*M*±SD). The homogeneity of the groups for the following parameters: number, lactation number (heifer vs. cow), age, weight, body condition score (BCS), cyclicity (cycling vs. anoestrus females), as well as the study of the distribution of frequency of ovulation by time period (48–72 h, 72–96 h, 96–120 h) were tested using a chi-square test. The fixed effects of the variables eCG (administration or not), category (heifer or cow) and state of cyclicity when the CIDR® was inserted (cycled or anoestrus) and covariables dose administration, age, weight and body condition, on the rate of growth and diameter of the follicle, were studied using a generalised linear model (GLM). The use of logistical regression allowed the effects of these same variables (treatment, lactation number, cyclicity) and covariables (dose, age, weight, body condition) on the time of ovulation to be analysed. All calculations were carried out using SAS software (version 9.1).

Fig. 1 Experimental protocol



Results

The characteristics of the animals in the study are presented in Table 1. No significant difference was observed between the groups for each parameter analysed (P>0.05).

Oestrus and follicle characteristics

Animals treated using 300 or 600 IU of eCG showed a significant increase in the percentages of observed oestrus (P<0.02) and ovulation (P<0.01). In the control group and treated animals, percentages of oestrus and ovulation were, respectively, 50 vs 79.8 % and 73.1 vs 94.7 % (Table 2).

Follicle growth rate was noted to be significantly (P<0.05) higher in animals treated with eCG (1.0±0.4 mm/day) than in the control group (0.9±0.4 mm/day). It appeared lower after administration of 600 IU of eCG when compared to injection of doses between 300 and 500 IU (Table 2).

The diameter of the preovulatory follicle varied from 5.8 to 12.2 mm. This diameter was significantly larger (P<0.05) in animals treated with 300 IU (10.1±1.4 mm) than in untreated animals (9.3±1.2 mm). Administration of eCG doses higher than 300 IU did not demonstrate significantly different rates of follicle growth or preovulatory follicle diameter (P>0.05) between the treated animals and the control group. However, a tendency to reduce the rate of follicle growth (P=0.07) as the dose of eCG increased was observed (Table 2).

Time of ovulation

The average intervals between the eCG injection, the start of oestrus, the end of oestrus and ovulation were, respectively, 83.7±14.4 h, 33.6±11.1 h and 23.0±11.8 h in the control group and 79.7±11.9 h, 30.1±10.1 h and 21.5±10.7 h in animals treated with eCG. No significant difference (P>0.05) was observed between the control group and the animals treated with eCG. However, lower values were noted in animals treated with 600 IU of eCG (Table 3).

All observed ovulation took place within 48 to 120 h after removal of the CIDR®. Both in the control group and the treated animals, the percentage of ovulation observed between 72 and 96 h after removal of the CIDR® was significantly (P<0.05) higher than 48 to 72 h or 97 to 120 h after removal. The percentage of ovulation observed in the animals treated with 600 IU of eCG was higher 48 to 72 h after removal than 73 to 96 h or 96 to 120 h (Table 4).

There was no significant (P>0.05) difference in average ovulation percentages observed at 48 to 72 h, 73 to 96 h and 97 to 120 h after CIDR® removal between the treated animals and the control group.

The induction rate corresponds to the percentage of females presenting standing heat behaviour in comparison with the total number of animals. Growth rate was determined between day 0 and ovulation.

Difference threshold includes the following: no difference, P>0.05; tendency towards difference, P=0.05; significant difference, P<0.05; and very significant difference, P<0.01.

Table 1 Characteristics of the animals in the study (n=120)

Traits	Controls	300 IU	400 IU	500 IU	600 IU	RMSE	*P* value
Numbers	26	25	24	22	23	NA	0.990
Age (years)	4.6	4.4	4.5	4.4	4.2	0,6	0.160
Weight (kg)	224.8	225.4	217.9	220.8	211.2	19,5	0.364
BCS	2.4	2.5	2.3	2.5	2.4	NA	0.730
Parity and physiological status							
% heifers	65.4	52.0	45.9	60.9	39.1	NA	0.272
% of cycled females	80.8	92.0	91.6	91.3	95.7	NA	0.187

Means of traits per equine chorionic gonadotropin (eCG) dosage in trypanotolerant N'Dama cows treated with controlled internal drug releasing device

NA not applicable, *IU* International Unit (of eCG), *BCS* body condition score, *RMSE* root-mean-square error.

Table 2 Compared effects of eCG dose (0 to 600 IU) on oestral, follicle and ovulatory characteristics in female N'Dama

Traits	Groups					*P* value
	Controls	300 IU	400 IU	500 IU	600 IU	
Numbers	26	25	24	22	23	0.99
% of oestrus	50.0	84.0	70.8	81.8	82.6	0.02
% ovulation	73.1[a]	88.0[b]	91.7[c]	95.4[d]	100[e]	0.01
FGR (day 0–ovulation)	0.9±0.4[a]	1.1±0.5[b]	1.0±0.4[c]	1.1±0.4[d]	0.8±0.4[e]	0.02
OF diameter (mm)	9.3±1.2[a]	10.1±1.4[b]	9.3±1.5[a]	9.1±1.2[a]	8.6±1.2[a]	0.04

IU International Unit (of eCG), *FGR* follicle growth rate (mm), *OF* ovulating follicle

Means in the same line with different letters differ significantly ($P<0.05$)

The numbers followed by letters (in each ligne) indicate a significant difference.

Discussion

The number of animals ($n=120$) in our trial was significantly higher than that of other studies (Ralambofiringa 1978, Meyer and Yesso 1995). The average age of the animals was relatively low (4.4±0.6 years) because the average age between birth and first calving is between 3.5 and 4 years (Coulomb 1976). Average weight (221.2±20.0 kg) was also lower than that known for females in this breed (286.7±8.3 kg) (Coulomb 1976). Their body condition score (2.4±0.4) corresponded to that usually seen in females of reproductive age (Ezanno et al. 2005).

Table 3 Effect of eCG dose on the time of ovulation in female N'Dama

	Number of cows	Interval between eCG injection and ovulation (hours)	Interval between the start of oestrus and ovulation (hours)	Interval between the end of oestrus and ovulation (hours)
Controls	26	83.7±14.4 ($n=19$)	33.6±11.1 ($n=12$)	23.0±11.8 ($n=12$)
300 IU	25	80.4±11.2 ($n=22$)	33.5±11.2 ($n=20$)	23.5±11.5 ($n=20$)
400 IU	24	78.7±11.1 ($n=23$)	29.8±9.2 ($n=16$)	20.5±10.8 ($n=16$)
500 IU	22	81.8±11.9 ($n=21$)	31.4±10.0 ($n=17$)	21.7±10.8 ($n=17$)
600 IU	23	78.0±13.5 ($n=23$)	28.7±9.9 ($n=19$)	19.9±10.4 ($n=19$)
Average of treated animals		79.7±11.9 ($n=89$)	30.1±10.1 ($n=72$)	21.5±10.7 ($n=72$)
General average		80.4±12.4 ($n=108$)	31.0±10.3 ($n=84$)	21.3±10.5 ($n=84$)

No significant difference ($P>0.05$)

Injection of eCG (300 to 500 IU) induced a significant increase in follicle growth rate (1.0±0.4 to 1.1±0.5 mm) in comparison with the control group (0.9±0.4). This effect has been observed by other authors (Baruselli et al. 2004; Lopes et al. 2007). Injection of 400 IU of eCG in 90 Nelore heifers presented a preovulatory follicle growth rate of 1.1±0.1 mm/day, while this was 0.6 ±0.1 mm/day in the 87 animals in the control group (Sà Filho et al. 2010b). The same is true of Nelore cows treated with 400 IU of eCG: follicle growth rate was 1.5±0.1 mm/day compared to 0.56±0.2 mm/day in untreated cows (Sà Filho et al. 2010a). Animals injected with eCG (300 IU) present a greater ovulating follicle diameter (10.1±1.4 mm) than the control group (9.3±1.2 mm). Similar differences were observed after injection of 400 IU of eCG in Nelore cows (*Bos indicus*) (11.2 ±0.9 mm vs. 9.4±1.1 mm) (Sà Filho et al. 2010a) This is lower than the diameters observed by several authors after injecting 400 IU of eCG in Nelore cattle: 10.6±0.2 mm, 11.2±0.9 and 12.55±0.36 mm (Baruselli et al. 2004; Sà Filho et al. 2010a, c), or Senepol cattle: 13.6±0.4 mm (Alvarez et al. 2000). These differences could be attributed to speculation and therefore the different physiological statuses of the *B. taurus* breeds. In this breed, ovulation can be observed when the diameter of the follicle is close to 10 mm (Ginther et al. 1996; Sartori et al. 2001).

Table 4 Effect of eCG dose on distribution of intervals between treatment and ovulation in female N'Dama

Groups	% of females who ovulated within		
	48–72 h	73–96 h	97–120 h
Controls	26.3[a]	57.9[b]	15.8[a]
300 IU	13.6[a]	72.8[b]	13.6[a]
400 IU	21.7[a]	56.6[b]	21.7[a]
500 IU	14.3[a]	66.7[b]	19.0[a]
600 IU	56.5[c]	34.8[d]	8.7[e]
eCG average	27.0[a]	57.3[b]	15.7[a]
General Average	25.0[a]	57.4[b]	17.6[a]

The numbers followed by letters are significantly different to the threshold $P>0.05$

Dairy cattle respond in different ways to eCG administration. In the Holstein breed, the diameter of the ovulatory follicle in the control group and the group treated with an average of 400 IU of eCG injected upon removal of the CIDR® were, respectively, 13.1±0.6 mm and 14.7±0.6 mm. This difference was primarily observed in animals presenting a weaker body condition (Souza et al. 2009). Similarly, the effect of eCG is more obvious in cattle with higher milk production (Garcia-Ispierto et al. 2012) and those over the age of five (Bryan et al. 2010). Increasing the eCG dose (from 400 to 600 IU) results, in this case, in a reduction in the ovulatory follicle growth rate and follicle diameter. Similar effects were also reported in dairy cattle. Thus, after injection of 400 or 600 IU of eCG, the growth rate and diameter of the ovulatory follicle were, respectively, 3.9±0.6 mm/day and 14.5±1.1 mm, and 3.5±0.5 mm/day and 13.9±1.2 mm (Ferreira et al. 2013). It does not appear that a reduction in the diameter of the ovulatory follicle is a determining factor for infertility (Sartori et al. 2001; Perry et al. 2005) as long as a minimal value close to 10 mm is observed (Martinez et al. 1999; Sartori et al. 2001). Thus, in Senepol cattle (*B. taurus*) and Nelore cattle (*B. indicus*), ovulation of the follicle with a diameter less than 8.5 mm is considered as physiological (Sartori and Barros 2011). Similarly, in the Zebu Nelore, gestations have been observed after ovulation of follicles with a diameter less than 7.5 mm (Sá Filho et al. 2010c). However, reduction in the diameter of the ovulating follicle may be accompanied by progesteronaemia (Sartori and Barros 2011). An increase in the LH effect resulting from the injection of eCG (Sheldon and Dobson 2000; Rostami et al. 2011) induces the ovulation of follicles with a smaller diameter (Perry et al. 2005). This possibility explains the fact that, in our study, the increase in eCG dose is accompanied by an increase in the percentage of ovulations.

An average interval of 80.4±12.4 h was observed between removal of the CIDR® and ovulation. Injections of eCG had no effect on this interval. Intervals of 72.9±2.1 h and 70.5±2.7 h were observed after dairy cattle were treated with a CIDR® and 400 IU of eCG (Souza et al. 2009) and Nelore cattle treated using a norgestomet implant and 400 IU of eCG (Sà Filho et al. 2010a). Injection of 400 IU of eCG did not lead to significant differences in this interval in Nelore cows treated using progesterone, 74.2±4.0 h in treated cattle compared to 78.0±3.1 h in cows in the control group (Baruselli et al. 2004). Increasing the dose of eCG (from 400 to 600 IU) did not lead to significant differences in the interval between treatment and ovulation in Holstein cattle. This interval was, respectively, 82.9±2.2 h in cows in the control group and 78.5±2.3 h in cows treated with 400 IU and 70.8 h in cows treated with 600 IU (Ferreira et al. 2013). The majority of ovulations observed took place within 72 to 96 h after removal of the CIDR®. It is possible that injecting eCG increases synchronisation of ovulation, favouring the use of systematic inseminations (Souza et al. 2009; Garcia-Ispierto et al. 2012).

Conclusion

Our study has clarified the follicular and ovulatory characteristics of female N'Dama cattle treated for 7 days with progesterone (CIDR®) combined, at the end of treatment, with an injection of eCG and $PGF_{2\alpha}$. Injection of eCG contributes towards increasing the percentages of oestrus and ovulations observed. It has no effect on the intervals between treatment and ovulation. It nevertheless contributes towards greater synchronicity of the time of ovulation 72 to 96 h after the end of treatment, which is likely to favour the use of systematic single insemination 72 h or double insemination 66 and 84 h after removal of the CIDR®.

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Conflict of interest The authors declare that they have no competing interests.

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