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MULTIPLE SUPEROVULATIONS IN N'DAMA HEIFERS

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SUMMARY

Five N'Dama heifers were superovulated with follicle stimulating hormone (FSH-P or Folltropin) a total of six times each. The superovulations were carried out between ongoing experimental Trypanosoma congolense *infections. Twenty-four (80%) of the 30 superovulations had a good ovarian response with 21 (70%) producing an average of 2·7 ± 0·4 (mean ± s.e.m.) embryos. The highest embryo production was achieved at the third and fourth superovulation, after which both the number of embryos and their quality declined. The overall pregnancy rate after transfer into Boran* (Bos indicus) *cow recipients was 50·9%. The uteri of the heifers increased considerably in size throughout the six superovulations which made it difficult to flush some of the animals after the third superovulation. Embryo transfer technology is a useful breeding tool in N'Dama heifers and multiple superovulations can be carried out with success.*

INTRODUCTION

Ten N'Dama calves (five females and five males) were born at ILRAD in March and April 1984. They were the result of an embryo transfer project involving the transfer of frozen N'Dama embryos from The Gambia to Kenya, where the trypanotolerant animals were needed for studies on trypanotolerance (Jordt, Mahon, Touray, Ngulo, Morrison, Rawle and Murray, 1986). Eight of the N'Damas (five females and three males) were challenged five times with tsetse-transmitted *Trypanosoma congolense* between the ages of one and four years (Logan, Paling, Moloo and Scott, 1987). Five N'Damas (three females and two males) were challenged a sixth time with *T. congolense* at the age of four-and-a-half years (Williams, Naessens, Moloo and Scott, in preparation). Since more N'Dama calves were needed for these studies an embryo transfer project was initiated with the objective of producing as many pure-bred N'Damas as possible. The embryo transfer technology had not previously been used on N'Dama heifers and the project was therefore also used to evaluate their suitability as embryo donors.

MATERIALS AND METHODS

The 10 N'Damas were kept in one of ILRAD's covered concrete-floored units where they were fed concentrates (UNGA Ltd), hay and a mineral mixture (Skaj Ltd) and given an injection of vitamins A, D and E (Nord-Agrar Gmbh) three times a year.

The five females showed their first oestrus when they were two years old and subsequently displayed oestrus regularly, despite a series of trypanosome infections (Lorenzini, Scott, Paling and Jordt, 1987). Oestrous cycles were monitored on a regular basis by rectal palpation, as well as by measuring their

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blood progesterone level using an amplified enzyme immunoassay (Enzygnost, Hoechst Ltd).

The heifers were superovulated a total of six times between the ages of two-and-a-half and four-and-a-half years. The intervals between the superovulations ranged from two-and-a-half to six-and-a-half months, depending on the starting dates and duration of the ongoing trypanosome infections.

The first and second superovulations were done between the third and fourth and the fourth and fifth tyrpanosome infections respectively and three to four weeks after the therapeutic treatment (7 mg/kg diamizine aceturate, Berenil, Hoechst) at the end of each experimental period (Logan *et al.*, 1987). The third, fourth and fifth superovulations were carried out between the fifth and sixth trypanosome infection with the animals being treated with Berenil eight days after the third superovulation. The heifers ND3, ND4 and ND5, which were the only heifers infected with trypanosomes the sixth time, were not treated with Berenil before the sixth superovulation. At the time of superovulation, none of the animals had a lowered packed cell volume nor detectable parasitaemia.

All five females were on the hormonal treatment programme at the same time. Their oestrous cycles were synchronised by the insertion of a progesterone releasing intravaginal device (PRID, Abbott) from which the oestradiol capsule was removed, regardless of the stage of their oestrous cycle. Six days later the females were injected with 500 μg cloprostenol, a prostaglandin $F_{2\alpha}$ analogue (Estrumate, Coopers), and the PRID was removed 24 hours later. The heifers came into oestrus 48 to 72 hours after the PRID removal (Wilson, Parker, Foulkes and Saurer, 1986; Jordt and Lorenzini, 1988).

The follicle stimulating hormone FSH-P (Burns-Biotec) or Folltropin (Vetrepharm Inc) was used to superovulate the heifers. A total dosage of 28 mg to 40 mg of FSH-P or 25·2 mg Folltropin was injected, 10 to 11 days after the induced oestrus, in declining daily dosages over a four-day period (e.g. 2×5 mg, 2×4 mg, 2×3 mg 2×2 mg for 28 mg FSH-P); 48 hours after the initial follicle stimulating hormone administration, all heifers were injected with 750 μg cloprostenol, which induced oestrus 48 to 72 hours later (Jordt *et al.*, 1986).

At the first superovulation all five heifers were bred by natural service because no frozen N'Dama semen was available. They were brought together with a bull on the evening prior to what was expected to be the first day of their oestrus and remained with the bull for one to two days. At the next five superovulations the heifers were artificially inseminated 48, 60 and 72 hours after the Estrumate injection using two straws of semen at each occasion. The semen was collected from the N'Dama bulls by staff from the Central Artificial Insemination Station (CAIS), Kabete, Kenya, and processed at the CAIS laboratory.

Seven to eight days after oestrus/service the uteri were flushed for embryo removal using a two-way Foley catheter (Franklin Medical Ltd) and Ovum Culture Medium (Flow Laboratories). Afterwards, the donors received both an intrauterine infusion of 10 ml antibiotic (Pen and Strep, Duphar BV) and one injection of 500 μg cloprostenol. The embryos collected were examined under a stereomicroscope (Wild Heerbrugg Ltd) and the quality of the embryos determined using a four-category rating system as described by Lindner and Wright (1983); (A, excellent; B, good; C, fair; D, poor). The ovaries of the heifers were palpated on the day of flushing. If fewer than four corpora lutea were detected the heifer was considered to have responded poorly to the superovulatory

treatment (Lampeter, 1978). The embryos were transported in Ovum Culture Medium in disposable cat catheters (J. Kruuse A/S) at ambient temperature from ILRAD to ILRAD's breeding ranch, Kapiti Plains Estate, 50 km south-east of Nairobi where the Boran cow (*Bos indicus*) recipients were kept.

The Boran recipients were synchronised for oestrus using the same treatment as for the N'Dama donors so that they were in oestrus on the same day as the donors. To select the most suitable of the Boran cow recipients, the reproductive organs were palpated per rectum on the day of expected oestrus and again on the day of transfer, seven or eight days later. As an additional selection criterion their blood-progesterone levels were measured on the day of expected oestrus and again seven days later. Only recipients with plasma-progesterone levels of zero to one ng/ml at oestrus and five ng/ml or above seven days later were used (Jordt and Lorenzini, 1988).

The embryos were transferred surgically via a flank incision into the uterine horn ipsilateral to the corpus luteum using a 10 μl dispenser (Scientific Manufacturing Industries). Two months later the recipients were palpated rectally for diagnosis of pregnancy.

RESULTS

At the first superovulation it was difficult to pass the Foley catheter through the small cervices of the N'Dama heifers. Even a small catheter (CH 14) could not be passed through the cervix of ND1 and this heifer was therefore not flushed. The size of the cervices and uteri increased after each superovulation, which made it easy to flush the heifers at the second and third superovulation, but as the uteri continued to enlarge it became increasingly difficult to flush the heifers after the third superovulation. Furthermore, the narrow rectum of the relatively small heifers (shoulder height, 111 ± 3 cm [mean $\pm$ s.d.], and weight, 353 ± 23 kg [mean $\pm$ s.d.], at four years of age) restricted the hand-arm movement of the operator when manipulating their uteri. Heifers ND2 and ND4 were particularly difficult to flush because their uteri had become thin-walled with very little tone and so large that a complete examination of the uterine horns was impossible. It was therefore difficult to ascertain that the Foley catheter was correctly positioned.

The ovarian response was not influenced by the dosage or the type of follicle stimulating hormone used, with 80% (24/30) of the superovulations producing a good ovarian response. Only heifer ND3 seemed, at the fifth superovulation to benefit from the use of Folltropin, which was therefore also used on this heifer at the sixth superovulation (Table I).

The ovarian response and the embryo production were not affected by the concurrent trypanosome infections present at the time of the third and sixth superovulations, which were carried out prior to the Berenil treatment.

The results from the six superovulations cannot be directly compared because of the limited number of donors. But it is interesting to note that the embryo production peaked at the third (15 embryos) and fourth (12 embryos) superovulations and that the number of good-quality embryos (A plus B) was higher for the first four superovulations than for the last two (86% *vs.* 54%; Table II). The total number of embryos produced varied considerably among the heifers. ND1, ND2 and ND3 produced fewer embryos (six, eight and five respectively) than ND4 and ND5 (23 and 15 respectively; Table I).

TABLE I

Individual embryo-ova production in N'Dama heifers

Date	Dosage	ND1 VE	DE	UFO	ND2 VE	DE	UFO	ND3 VE	DE	UFO	ND4 VE	DE	UFO	ND5 VE	DE	UFO	TVE
Nov.	28 mg	Not															
1986	FSH-P	flushed			1	0	0	0	0	4	5	0	0	2	1	0	8
April	32 mg																
1987	FSH-P	1	0	0	1	0	0	0	0	0	3	0	1	4	0	0	9
Aug.	36 mg																
1987	FSH-P	2	0	0	5	0	1	0	0	1	3	0	0	5	1	2	15
Nov.	40 mg																
1987	FSH-P	3	0	0	1	1	0	1	0	0	5*	0	0	2	0	0	12
Feb.	25·2 mg																
1988	Folltropin	0	0	0	0	0	0	3	0	1	3	0	1	0	0	0	6
Sept.	36 mg																
1988	FSH-P	0	0	0	0	0	0	—	—	—	4	0	2	2	0	2	
	25·2 mg																7
	Folltropin	—	—	—	—	—	—	1	0	1	—	—	—	—	—	—	
Total		6	0	0	8	1	1	5	0	7	23	0	4	15	2	4	57

* No pregnancies were produced from excellent quality embryos. VE—Viable embryos; TVE—Total number of viable embryos; DE—Degenerated embryos; UFO—Unfertilised ova.

The overall pregnancy rate was 50·9% (29/59). The pregnancy rate for each of the six superovulations ranged from 28·6% to 77·7%, with the sixth superovulation having both the lowest pregnancy rate and the highest proportion (57%) of poor-quality (C plus D) embryos (Table II).

At the fourth superovulation, an unexpected low pregnancy rate of 33·3% was experienced as the consequence of the fact that none of the five excellent-

TABLE II

Embryo production, embryo quality and pregnancies in relation to number of superovulations in N'Dama heifers

Superovulation	DGR	DPE	TVE	EPEP	AEPS	Embryo quality A	B	C	D	Pregnancies
First	4	3	8	2·6 ± 1·3	1·6 ± 0·9	6	2	0	0	4 (50·0%)
Second	4	4	9	2·2 ± 0·3	1·8 ± 0·7	4	4	0	1	7 (77·7%)
Third	5	4	15	3·7 ± 0·8	3·0 ± 0·9	8	5	2	0	8[a] (53·3%)
Fourth	3	5	12	2·4 ± 0·7	2·4 ± 0·7	9	0	2	1	4 (33·3%)
Fifth	4	2	6	3·0 ± 0·0	1·2 ± 0·7	3	1	1	1	4[b] (66·6%)
Sixth	4	3	7	2·3 ± 0·8	1·4 ± 0·7	1	2	2	2	2 (28·6%)
Total	24	21	57	2·7 ± 0·4	1·9 ± 0·3	31	14	7	5	29 (50·9%)
percentage	80	70								

[a] A foetus aborted four months after the transfer and a calf was born prematurely, and died, eight months after the transfer.
[b] A foetus aborted four months after the transfer.
DGR—Donors with a good ovarian response.
DPE—Donors producing embryos.
TVE—Total number of viable embryos.
EPEP—Embryos per embryo producer (mean ± s.e.m.).
AEPS—Average embryo production per superovulation (mean ± s.e.m.).

embryos from heifer ND4 produced a pregnancy (Table I). At the first superovulation heifer ND3 produced four unfertilised ova only. Unfertilised ova are a normal finding in superovulated cattle but the bull that serviced heifer ND3 did not produce any good quality semen at the semen collections and this perhaps explains the lack of fertilisation (Table I).

Twenty-four N'Dama calves (10 females and 14 males) have been born so far; 21 of these were produced within one year.

DISCUSSION

The average embryo production per superovulation (AEPS) is a good indication of the abilty of a group of cows/heifers to produce embryos. The AEPS of 1·9 embryos for the 30 superovulations is quite satisfactory but lower than averages reported for various *Bos taurus* breeds in Europe (Bak, Greve and Schmidt, 1987, AEPS: 4·1; Nibart and Thibier, 1987, AEPS: 3·1). Compared with other published results on African breeds, this AEPS is higher than that of N'Dama cows (Jordt *et al.*, 1986, AEPS: 1·3) and Baoulé cows (Bianchi, Chicoteau, Cloé and Basssinga, 1986, AEPS: 0·2) but lower than that of Boran cows (Jordt and Lorenzini, 1988, AEPS: 2·2). The degree of good ovarian response (80%) and the number of superovulations producing embryos (70%) were both higher in this group of heifers than in the N'Dama cows (54·2% and 29·2%, respectively), the Baoulé cows (71% with good ovarian response) and the Boran cows (75·5% and 59·4% respectively). However, the embryo production per embryo producer (EPEP) of 2·7 embryos was lower than that of N'Dama cows (EPEP: 4·3) and Boran cows (EPEP: 3·7). These results on N'Dama cows and heifers indicate that the ability to produce more embryos per embryo producer might develop after the first calving.

Donors that are selected to enter an embryo transfer programme must have displayed oestrus regularly. When Boran cows and heifers were infected with *T. congolense,* their oestrous cycles ceased (Lorenzini *et al.*, 1987; Llewelyn, Munro, Luckins, Jordt, Murray and Lorenzini, 1988); whereas the trypanotolerant N'Dama heifers continued to cycle throughout the ongoing trypanosome infections (Lorenzini *et al.*, 1987) and therefore responded positively to the superovulatory treatments. The fact that the ovarian response and the embryo production were not influenced by trypanosome infection at the third and sixth superovulations shows the breeding potential of the N'Damas in trypanosomiasis affected areas.

Although 36 mg FSH-P at the third superovulation produced the highest number of embryos, it cannot be concluded that this is the optimum dosage for superovulation in N'Dama heifers because the use of 36 mg FSH-P on four animals at the sixth superovulation did not produce a similar result. It is possible that the heifers would have achieved an optimum embryo production potential at the third superovulation whatever dosage was used. We believe, however, that the dosage of 36 mg FSH-P is a good choice when superovulating N'Dama heifers.

The quality and quantity of embryo production declined after the fourth superovulation. Repeated superovulations in Brahman cows also had a detrimental effect on the embryo parameters, especially after the fourth superovulation (Bastidas and Randel, 1987). However, no significant difference in embryo production was found among Boran donors superovulated one to five times (Jordt

and Lorenzini, 1988). Reports on Holstein cows mention a decrease in fertilisation rate after five superovulations but the ability of this breed of cows to respond repeatedly to a follicle stimulating hormone was consistent throughout 10 superovulations (Hasler, McCauley, Schermerhorn and Foote, 1983).

We cannot immediately explain why the uteri developed so dramatically in size since we have not experienced this before and have not found it reported in other breeds. Endometritis can produce similar changes but it is unlikely this was the cause since the donors received a preventive treatment after each flushing. Furthermore, the flushing medium used was always found to be clear. Perhaps the uterine changes were side effects of the hormonal treatments.

The overall pregnancy rate of 50·9% in the Boran cow recipients is satisfactory but lower than the 63·3% reported for Borans (Jordt and Lorenzini, 1988). The explanation is that, in this work, all embryos were transferred as enough recipients were ready for an implantation, but only one out of five D-quality embryos produced a pregnancy. In normal practice several of these embryos would not have been transferred due to their poor quality. Furthermore, five excellent quality embryos from ND4 at the third superovulation produced no pregnancy, where normally three pregnancies would have been expected.

Because of the decreased embryo production, the reduced embryo quality and the uteri proving increasingly difficult to flush, we decided not to superovulate more than six times. The heifers therefore entered a normal breeding programme two months after the sixth superovulation.

Our findings suggest that N'Dama heifers should be superovulated only four times, after which they must be allowed to go through a normal pregnancy before they are superovulated again.

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REFERENCES

BAK, A., GREVE, T. & SCHMIDT, M. (1987). Embryo transfer in Denmark: Contemporary results obtained in a national programme. Report from the 3rd Scientific Meeting of the European Embryo Transfer Association, Lyon, pp 17–25.

BASTIDAS, P. & RANDEL, R. D. (1987). *Theriogenology,* **28,** 827–835.

BIANCHI, M., CHICOTEAU, P., CLOÉ, C. & BASSINGA, A. (1986). *Revue d'Elevage et de Médecine Vétérinaire des Pays Tropicaux,* **39,** 139–144.

HASLER, J. F., MCCAULEY, A. D., SCHERMERHORN, E. C. & FOOTE, R. H. (1983). *Theriogenology,* **19,** 83–99.

JORDT, T., MAHON, G. D., TOURAY, B. N., NGULO, W. K., MORRISON, W. I., RAWLE, J. & MURRAY, M. (1986). *Tropical Animal Health and Production,* **18,** 65–75.

JORDT, T. & LORENZINI, E. (1988). *Theriogenology,* **30,** 355–367.

LAMPETER, W. W. (1978). *Zuchthygiene,* **13,** 1–4.

LLEWELYN, C. A., MUNRO, C. D., LUCKINS, A. G., JORDT, T., MURRAY, M. & LORENZINI, E. (1988). *British Veterinary Journal,* **144,** 379–387.

LINDNER, G. M. & WRIGHT, R. W. (1983). *Theriogenology,* **20,** 407–416.

LOGAN, L. L., PALING, R. W., MOLOO, S. K. & SCOTT, J. R. (1987). Comparative studies on the responses of N'Dama and Boran cattle to experimental challenge with tsetse-transmitted *Trypanosoma congolense.* In: Proceedings of a meeting on Livestock Production in Tsetse Affected Areas of Africa: ILCA/ILRAD, Nairobi, pp 152–160.

LORENZINI, E., SCOTT, J. R., PALING, R. W. & JORDT, T. (1987). The effects of *Trypanosoma congolense* infection on the reproductive cycle of N'Dama and Boran heifers. In: Proceedings of a meeting on Livestock Production in Tsetse Affected Areas in Africa: ILCA/ILRAD, Nairobi, pp 168–173.

NIBART, M. & THIBIER, M. (1987). Le Transfert Embryonnaire en France. Report from the 3rd Scientific Meeting of the European Embryo Transfer Association, Lyon, pp 27–39.

WILSON, G. D. A., PARKER, B. N. J., FOULKES, J. A. & SAURER, M. J. (1986). *British Veterinary Journal,* **142,** 47–51.

SUPERVULATIONS REPETEES CHEZ DES GENISSES N'DAMA

Résumé—Cinq génisses N'Dama ont été supervoluées six fois au total avec de la FSH (FSH-P ou Follotropine ND). Les superovulations ont été réalisées pendant le déroulement d'infections expérimentales à *Trypanosoma congolense.* 24 des 30 superovulations (soit 80 p. 100) ont été accompagnées d'une bonne réponse ovarienne, 21 d'entre elles, (soit 70 p. 100), produisant une moyenne de 2,7 + ou − 0,4 embryons (moyenne + écart type). La plus forte production d'embryons a été obtenue lors des 3e et 4e superovulations. Par la suite, le nombre des embryons et leur qualité ont simultanément diminué. Le taux de gestation global après transfert sur des vaches receveuses de race Boran a été de 50, 9 p. 100. L'utérus des génisses a augmenté considérablement de taille au cours des six superovulations successives, ce qui a rendu difficile la collecte d'embryons sur quelques animaux après la 3e. Le transfert d'embryon est une méthode utile de reproduction chez les génisses N'Dama et les superovulations répétées peuvent être conduites avec succès.

SUPEROVULACION MULTIPLE EN VAQUILLAS N'DAMA

Resumen—Se superovularon cinco vaquillas N'Dama seis veces cada una, con hormona folículo estimulante (FSH-P O Foltropin). Las superovulaciones se llevaron a cabo en medio de un trabajo experimental con *Trypanosoma congolense.* Veinticuatro (80%) de las 30 superovulaciones tuvieron una buena respuesta ovárica, con 21 (70%) produciendo un promedio de 2.7 ± 0.4 (mean $\pm$ s.e.m.) embriones. La mejor producción de embriones ocurrió a la tercera y cuarta superovulación, después de la cual, ambos, el número de embriones y la calidad declinaron. La tasa de preñez total, despues de la transferencia a ganado Boran (*Bos indicus*) fue de 50.9%. El tamaño uterino de las vaquillas aumentó considerablemente, a través de las seis superovulaciones, lo cual dificultó la recolección de embriones mediante lavado uterino, después de la tercera superovulación.

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