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EMBRYO PRODUCTION FROM REPEATED INDUCTION OF OVULATION IN GIGANTE DE ESPAÑA DOES

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ABSTRACT

The aim of this work was to assess the embryo production potential of the Gigante de España breed induced to ovulate with GnRH and mated naturally. One hundred and ninety six Gigante de España does were used as embryo donors after successive treatments for induction of ovulation with 20 mg of GnRH. All treated donors ovulated, with a mean ovulation rate of 8.6 corpora lutea. Embryos were collected in a stage of precompacted morula from 91% does, with an average of 7.0 recovered and 6.3 viable embryos per donor. The number of the ovulation treatment did not influence ovulation rate. However, the process seemed to become less efficient beyond the third treatment comparing to the second or third ones, either in terms of recovered (5.1 vs 7.4 and 7.0, respectively; $P<0.05$) or viable embryos (4.4 vs 6.8 and 6.5, respectively; $P<0.05$). More than 21 recovered and 19 viable embryos can be obtained from donor does receiving the 3 first ovulating treatments.

INTRODUCTION

The Gigante de España breed was firstly described in 1921. It's a local breed with good growth and maternal characteristics, that was the base of many Spanish rabbit farms until 1960. Nevertheless, the evolution of the rabbit breeding and the appearance of competitive new breeds (New-Zealand and Californian), were the cause of the progressive disappearance and almost extinction of the Gigante de España breed; however, the Department of Animal Production of the Veterinary Faculty of Zaragoza (Spain), undertook his recovery since 1984 from a limited population size (Sierra and López, 1990).

Gigante de España does are characterised by a percentage of pregnant and delivered female of 84 and 79 % respectively, with a prolificacy of 8.2 pups born and 7.7 live born (Sierra and López, 1990). After induction of ovulation with GnRH, 10.4 corpora lutea and 6.8 recovered embryos per donor were recorded (López et al., 1993).

In fact, the interest of the preservation of the breed permits to protect its cultural and historic heritage, and to preserve some useful genes in order to the diffusion of the breed in commercial farms. On the other hand, the perspective that this breed could constitute a bank of genes susceptible to be used in the future in the formation of animal groups, and the development of artificial insemination and embryo production and cryopreservation, converts its preservation in an important objective.

In our knowledge, no studies have been performed in the literature concerning the surgical collection of rabbit embryos after successive laparotomies, as the embryo collection of rabbits has been done exclusively after slaughtering the female. On the other hand, repeated surgically embryo collection procedures have been successfully used in ewes, although exteriorisation of the reproductive tract often leads to the formation of post-operative adhesions inducing a reduction in embryo recovery after repeated surgery (Torres and Sevellec, 1987).

The aim of this work was to assess the capacity of production of surgically recovered embryos of Gigante de España does naturally mated and induced to ovulate with GnRH. The study was performed over five consecutive years (1995-1999).

MATERIAL AND METHODS

The experiment was conducted at the experimental farm of the Veterinary Faculty of Zaragoza University (Spain), which meets the requirements of the European Community Commission (1986) for Scientific Procedure Establishments. All Gigante de España females were housed individually in metal cages with environmental conditions of 16 hours light a day and fed ad libitum. A total of 196 ovulating treatments were performed to embryo recovery. Females were naturally mated by New-Zealand bucks.

Twenty mg of GnRH (Fertagyl, INTERVET, Holland) were given i.m immediately after natural mating. Embryos were surgically collected 68 hours post-mating by ventral midline laparatomy. Anaesthesia was induced first by administering an intramuscular injection of 0.2 ml xylacine (2 %), followed by an intravenous injection sodium tiopental (20 mg/ml) (Thiobarbital, Braun Medical, Spain) 15 min later in the marginal ear vein, according to Abecia and Forcada (1989). A local anaesthesia was also induced administering a subcutaneous injection of lidocaine chlorohydrate.

Embryos were recovered by separate perfusion of the oviducts (10 ml) with pre-warmed (37º C) phosphate-bufferd saline (PBS) (Sigma, St. Louis, MO, USA) suplemented with an antibiotic solution of penicillin and streptomycin and sodium pyruvate 0.3 mM. The oviducts were flushed fom the utero-tubal junction to the fimbria. The recovered embryos were washed (PBS) and selected morphologically. The number of corpora lutea and the number of recovered embryos were recorded. The embryos were evaluated under a stereo-microscope at a magnification of 20-40 X, and classified by their stage of development, morphological apperance and quality (Fernando *et al*. 2000). Only selected embryos without any imperfections and with spherical shape were deemed as viable in this experiment.

Immediately after the surgical collection, the reproductive tract was washed with heparin solution (2.5 %) in saline before closure in order to minimize the post-operative development of abdominal adhesions. At the end of the laparotomy, the females were injected i.m. with 1 ml subcutaneous injection of an antibiotic solution and 0.2 ml of prostaglandin (Prosolvin, INTERVET, Holland). The females were given the same ovulatory treatment up the fourth times at intervals of at least 50 days.

Means (± SEM) were calculated for ovulation rate, numbers of recovered and viable embryos per donor and then compared by analysis variance to evaluate the difference between ovaries. The effect of the year, season and the number of the ovulatory treatment on the same doe on these variables was compared using the GLM procedure of the Statistical Analysis System (SAS, 1988).

RESULTS AND DISCUSSION

Mean ovulation rate recorded from Gigante de España does was 8.61 ± 0.15 corpora lutea with no significant differences between the two ovaries. All treated donors ovulated at least from one of the two ovaries (Table1). Percentage of ovulating does was similar to that reported by Théau-Clement *et al*. (1990) injecting GnRH immediately after the artificial insemination of receptive females slaughtered 14 days after mating (95%), and by López et *al*. (1993) using the same protocol and breed that in the present study (98%). On the other hand, ovulation rate recorded in Gigante de España does was similar to that obtained by López et *al*. (1993) using the same breed and experimental protocol (8.6 corpora lutea), but was lower to that reported by Abecia (1989) in the same experimental conditions, with an average of 10.7 and 10.4 corpora lutea per Hybrid and Gigante de España female, respectively.

Table 1. Average results of surgical embryo collection from Gigante de España does receiving 20 mg of GnRH i.m immediately after natural mating.

No. of ovulatory treatments	196
No. of donors ovulating from the left ovary	193
Ovulation rate (? ± SEM)	4.32 ± 0.10
No. of donors ovulating from the right ovary	194
Ovulation rate (? ± SEM)	4.40 ± 0.10
No. of donors ovulating from at least one ovary	196
Ovulation rate (? ± SEM)	8.61 ± 0.15
No. of left oviducts which their embryos were collected	165
No. of right oviducts which their embryos were collected	162
No. of donors which their embryos were collected	179 (91.3 %)
No. of embryos collected /female (? ± SEM)	7.04 ± 0.23
No. of viable embryos /female (? ± SEM)	6.32 ± 0.23

Embryos were collected in a stage of precompacted morula from 179 does (91.3%), with an average of 7.04 ± 0.23 embryos (Table 1). The number of recovered embryos per doe was similar to that obtained by López *et al*. (1993) in the same breed, with an average of 6.8 collected embryos per doe, but was lower to that reported by Vicente and García-Ximénez (1991), who recorded 9.7 embryos per doe in Hybrid females slaughtered 64-66 hours post-mating. The percentage of collected embryos in relation to the ovulation rate recorded in the present study was 75 % for the 196 does. Mean number of viable embryos was 6.32 ± 0.23 per doe, (90 % in relation to recovered embryos). Those embryos classified as non viable were non-fertilised (28 %), in an earlier stage of development than morula (23 %), degenerate (23 %) or had a non-spherical shape (26 %).

Table 2. Effect of the number of the treatment with 20 mg of GnRH on ovulation rate and embryo production of Gigante de España does (Means ± SEM)

No. of treatment	1	2	3	4
N. of corpora lutea	8.7 ± 0.2	8.7 ± 0.2	8.5 ± 0.4	7.9 ± 0.7
N. of donors (1)	71	65	29	9
N. of recovered embryos	6.8 ± 0.4^{ab}	7.4 ± 0.4^{a}	7.0 ± 0.5^{a}	5.1 ± 1.1^{b}
N. of donors (2)	60	59	29	9
N. of viable embryos	5.8 ± 0.4^{ab}	6.8 ± 0.4^{a}	6.5 ± 0.6^{a}	4.4 ± 1.0^{b}
N of donors (2)	60	59	29	9

a, b different superscripts between column indicate differences at least of $P<0.05$.
(1): Number of donors which ovulated at least from one ovary. The total number was 174; the number of treatment could not be identify in the remaining 22 does.
(2): Number of donors which their embryos were collected at least from one ovary.

No significant effect of year or season on the studied parameters (ovulation rate, number of collected and viable embryos) was detected, and data were pooled in order to test the effect of the ovulatory treatment number an ovarian response and embryo production (Table 2).
The number of the ovulatory treatment did not influence ovulation rate, with a number of

corpora lutea among 7.9 (fourth treatment) and 8.7 (first and second treatments) (Table 2). However, the process seemed to become less efficient in the fourth treatment, with a significant reduction of the number of recovered (5.7 vs 7.4 and 7.0 after the second and third treatments; $P<0.05$) and viable embryos (4.4 vs 6.8 and 6.5, respectively; $P<0.05$). Results of Table 2 also show that it is possible to obtain more than 21 recovered embryos and 19 viable embryos from donor does receiving the 3 first ovulatory treatments.

In conclusion, results of the present study indicate the possibility to obtain a high number of embryos from Gigante de España does, after repeated induction of ovulation with GnRH. Therefore, the methodology used could be an efficient tool in order to preservate and improve a selected genotype of limited population size.

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