EMBRYO RECOVERY UNDER ANAESTHESIA AFTER hCG OR GnRH TREATMENTS IN THE RABBIT AND SURVIVAL WHEN A REDUCED NUMBER OF EMBRYOS IS TRANSFERRED

LOPEZ Marina , FORCADA F., RODRIGUEZ J.A., MARTIN Maria, ZARAGAZA L.

Departamento de Producción Animal y Ciencia de los Alimentos
Faculdad de Veterinaria, Universidad de Zaragoza,
177, Miguel Servet. 50013 ZARAGOZA - Spain

SUMMARY : Ovulation rate and number of 72h post-mating recovered embryos were studied in nulliparous Spanish Giant does treated with an intravenous injection of 25 IU hCG (n=22) or 20 μg GnRH (n=20) intramuscularly after coitus. Embryos were recovered under anaesthesia by separate perfusion of the oviducts and uteri. One of the GnRH–treated does did not ovulate. No significant differences between groups were found either in the ovulation rate or in the number on recovered embryos per donor doe (8.5 ± 0.2 and 7.3 ± 0.2 vs 8.6 ± 0.4 and 6.8 ± 0.9 respectively for hCG and GnRH treated animals). After transfer into the uteri of synchronized Solaf commercial hybrid recipients, the lower number of embryos transferred per doe (5.9 vs 11.2) did not affect to the percentage of pregnant does (67 vs 75 % respectively), but increased the percentage of young born per pregnant doe (59 vs 41 % respectively) (P<0.05). Plasma progesterone levels in the recipients were higher in pregnant does (at least P<0.05) from Day 9 post–mating to the last blood sample recovered (Day 21), although the progesterone pattern in the non–pregnant ones indicated that the induction of ovulation was effective.

The results indicate the adequate embryo recovery rates obtained from Spanish Giant does under anaesthesia and the good efficiency after transfer of a reduced number of embryos per recipient doe.

RESUME : Transfert d'embryons chez la lapine anesthésiée, après traitement par hCG ou GnRH, et taux de survie lors du transfert d'un nombre réduit d'embryons.
Le taux d'ovulation et le nombre d'embryons retrouvés 72 heures après l'accouplement ont été étudiés chez 42 lapines primipares de race Géant d'Espagne traitées par injection intraveineuse de 25 UI d'hCG (n=22) ou intramusculaire de 20 μg GnRH (n=20) après l'accouplement. Les embryons ont été récupérés par perfusion séparée de l'oviducte et de l'utérus. Une des lapines traitées par GnRH n'a pas ovulé. Il n'y a pas de différences significatives entre les 2 groupes pour ce qui concerne le taux d'ovulation ou le nombre d'embryons retrouvés par lapine donneuse (8,5 ± 0,2 et 7,3 ± 0,2 vs 8,6 ± 0,4 et 6,8 ± 0,9 respectivement pour les animaux traités par hCG et GnRH). Après transfert dans l'utérus de receveuses hybrides commerciales Solaf synchronisées, le plus petit nombre d'embryons transférés par lapine (5,9 vs 11,2) n'a pas affecté le pourcentage de lapines gestantes (67 vs 75 % respectivement), mais a augmenté le pourcentage de lapereaux nés par lapine gestante (59 vs 41 % respectivement) (P<0.05). Le taux de progestérone plasmatique des receveuses était plus élevé chez les lapines gestantes (au moins P<0.05) à partir du 9ème jour après l'accouplement et jusqu'au dernier prélèvement de sang au 21ème jour, bien que le taux de progestérone chez les lapines non gestantes indique que l'ovulation était effectivement induite.
Les résultats montrent un taux de recouvrement d'embryons satisfaisant à partir de lapines Géant d'Espagne, sous anesthésie, et un bonne efficacité après transfert d'un nombre réduit d'embryons par lapine receveuse.

INTRODUCTION

Rabbit embryos are widely used in experimental embryology and reproductive medicine as well as in genetic experiments. Normal embryos are obtained when hCG is used to induce ovulation from donor does (VICENTE and GARCIA, 1991) although the treatment can accelerate their oviductal transit (BOURGADE and HALBERT, 1988 ; VICENTE and GARCIA, 1991). Endovenous doses of 10–25 IU of hCG induce ovulation even in does which are not receptive to the buck (HULOT et al., 1988). However, non–receptive does ovulating in response to hCG injection are characterized by a lower probability of successful pregnancy (PLA et al., 1986 ; MOODY and McNITT, 1988). Synthetic GnRH has been commonly used to induce ovulation after artificial insemination and to provoke a physiological number of ovulations in donor does (CARNEY and FOOTE, 1990), the probability of ovulation induction being higher when receptive does are used (THEAU–CLEMENT et al., 1990). Maximal survival rates in rabbits after embryo transfer are obtained when the donor and recipient are synchronized (CHANG, 1950 ; TECHAKUMPHU et al., 1987), and the embryonic mortality is increased when a higher number of embryos is transferred (HUIQING et al., 1987). Maternal recognition of pregnancy occurs on Day 12 after mating (NOWAK and BAHR, 1983).

The rabbit conceptus prolongs the life of the corpora lutea probably by a change in ovarian responsiveness to PGF-2α (MARCINKIEWICZ *et al.*, 1992). With the aim of preserving and improving the Spanish Giant breed, a program of study and diffusion has being developed since 1984 in the Veterinary Faculty of Zaragoza from a limited population size (SIERRA and LOPEZ, 1990). The present experiment was designed in order to study the ovulation rate and recovery of embryos from hCG or GnRH treated Spanish Giant does under anaesthesia (to allow subsequent treatments on the same donor females) and to determine the rate of survival and plasma progesterone levels after their transfer into synchronized recipients in relation to the number of embryos received for each transferred doe.

MATERIALS AND METHODS

Animals

The study was conducted at the experimental farm of the Veterinary Faculty of the Zaragoza University, Spain. A total of 67 nulliparous (42 Spanish Giant and 25 Solaf hybrids) female rabbits were used, weighing 4,49 and 4,73 kg respectively.

All animals were maintained and fed in the same way. They were housed individually in metal cages with environmental conditions of 16 hours light a day and temperature 20°C. Commercial pellets and water were available *ad libitum*.

Induction of ovulation

Animals were allocated to three groups :
– hCG treated animals : Spanish Giant donor does (n=22) in which ovulation was induced by mating plus an intravenous injection of 25 IU hCG (Coriogan, OVEJERO, Spain).
– GnRH treated animals : Spanish Giant donors does (n=20) in which ovulation was induced by mating plus intramuscular injection of 20 μg GnRH (Fertagyl, INTERVET, Holland).
– Twenty–five Solaf recipient does, in which ovulation was induced by an intravenous dose of 25 IU hCG. Animals showing a pale vulva at that moment were excluded, since females with white vulva show a reduced sexual receptivity and a low probability of ovulation induction (PLA *et al.*, 1986 ; MOODY and McNITT, 1988 ; FORCADA and ABECIA, 1990).

Embryo recovery

Embryos were surgically collected 72h post coitus by ventral midline laparotomy. Anaesthesia for both donor and recipient does was induced by first administering an intramuscular injection of 30 mg xylacine (Rompu, BAYER, Germany), followed 5–10 min later by an intravenous infusion of 1,5 %

solution of sodium thiopental (Pentothal, ABBOTT, France) in the marginal ear vein.

Embryos were recovered by separate perfusion at 37°C of the oviducts (5 ml) and uteri (7 ml) with phosphate–buffered saline (PBS, IMV, France) supplemented with 2 % of BSA. The oviducts were flushed from the utero–tubal junction to the fimbria. Each uterus was also flushed in reverse introducing a silastic catheter into the uterine lumen about 2 cm from the utero–tubal junction, while the flushing medium was injected at the base of uterus. The recovered embryos were washed (PBS) and selected morphologically, and their location (in the oviduct or uterus) was recorded.

Embryo transfer

The recipient does were synchronized with the donor ones by induction of ovulation at the same time, 72h before transfer. The transfer medium was the same PBS used to collect embryos from the donor. In order to evaluate the rate of embryo survival with the aim of optimizing the embryo transfer, each recipient doe received 6 (5 – 7) or 12 (10–13) embryos, half of them in each uterine horn. Consequently, groups of 3-6 embryos were aspirated into small plastic straws (IMV, France) with 25 μl of medium and isolated with two air bubbles. Transfer was carried out no later than 1h after recovery from the donor does by ventral midline laparotomy, and the embryos were transferred to the uterus by puncture near the utero–tubal junction.

The ovaries were not explored in the recipient does, and the presence and persistency of corpora lutea were assessed by the plasma progesterone concentrations. Samples of blood were collected every 3 days from the induction of ovulation (Day 0) up to Day 21 of the supposed pregnancy , which was diagnosed by abdominal palpation 12 days after ovulation induction. Litter size at birth in pregnant recipient does was recorded.

Progesterone assay

Plasma progesterone concentrations were determined by radioinmuassay as described by FORCADA and ABECIA (1990). Inter and intra assay coefficients of variation were 7.7 and 4.7 % respectively.

Statistical analysis

The proportion of normal and abnormal embryos in the oviduct or uterus and the percentage of pregnant recipients and of pups born for pregnant does were compared by chi–square or Fisher tests according to the number of observations. Means ($\pm$ SEM) were calculated for ovulation rate, number of embryos recovered and viable (donor does), litter size and plasma progesterone concentrations (recipient does), and were then compared by variance analysis. A 2 x 2

Table 1 : Ovulation rate and number of recovered and viable embryos per donor doe (Means ± SEM)

Group	No. of does	Ovulation rate	No. of embryos	No. of embryos morphologically normal
hCG treated donor	22	8.5 ±0.2	7.3 ± 0.2	6.8 ± 0.3
GnRH treated donors [1]	19	8.6 ±0.4	6.8 ± 0.9	6.4 ± 0.8

[1]One doe did not ovulate.
No significant differences in any case

factorial analysis of variance was used to evaluate the effects of pregnancy and number of transferred embryos on plasma progesterone concentrations.

RESULTS

All does ovulated in th hCG treated group, and only one female injected with GnRH did not ovulate. No significant differences were found between groups with respect to the ovulation rate, the number of embryos recovered per donor doe and the number of embryos which were scored as being morphologically normal (Table 1).

In relation to embryo abnormalities, no significant differences were detected between groups. Only 7 % of the embryos were visibly abnormal (not fertilized, having a grossly irregular morphology or with a stage of development which did not correspond to the time of surgical recovery). The embryos scores morphologically normal were at the late morulae stage except for 8 blastocysts in each of the two studied groups.

The percentage of embryos recovered from the uterus was not statistically different between groups (Table 2).

Data corresponding to development *in vivo* after transfer of embryos from hCG or GnRH groups were pooled since the figures were very similar (Table 3). Eighteen of the 25 recipient does were diagnosed as being positive and became pregnant without significant differences between the two considered group in relation to the number of 72h postcoitus transferred embryos. The percentage of youngs born for pregnant does was higher when a reduced number of embryos (6 vs 12) were transferred (P<0.05), but no significant differences were found in the percentage of youngs born calculated for both pregnant and non-pregnant does (48.9 vs 31.3 % for 6 vs 12 transferred embryos).

Table 2 : Location (oviduct or uterus) and morphological evaluation of recovered embryos.

Group	No. of embryos in oviducts (%)	No. of embryos in uterus (%)	No. of embryos morphologically abnormal in oviduct (%)	No. of embryos morphologically abnormal in uterus (%)
hCG treated donors	153 (95.0)	8 (5.0)	9 (5.9)	2 (25.0)
GnRH treated donors	127 (97.7)	3 (2.3)	9 (7.1)	0 (0)
Total	*280 (96.2)*	*11 (3.8)*	*18 (6.4)*	*2 (18.2)*

No significant differences in any case.

Table 3 : Effect of the number of 72h post mating transferred embryos on the development *in vivo*.

Group	No. of recipient does	No. of embryos transferred [1]	No. of pregnant does (%)	No. of youngs born	Youngs born (%)[2]
6 embryos	13	5.9 ± 0.04	9 (69)	3.7 ± 0.41	58.9[a]
12 embryos	12	11.2 ± 0.09	9 (75)	4.7 ± 0.53	41.2[b]

[1] Means ± SEM
[2] Calculated for pregnant does.
[a,b] Percentage with different superscript in the same column differ P<0.05.

Figure 1 : Plasma progesterone concentrations (ng/ml) after induction of ovulation in pregnant (P) and non-pregnant (NP) recipient does receiving 6 or 12 embryos.
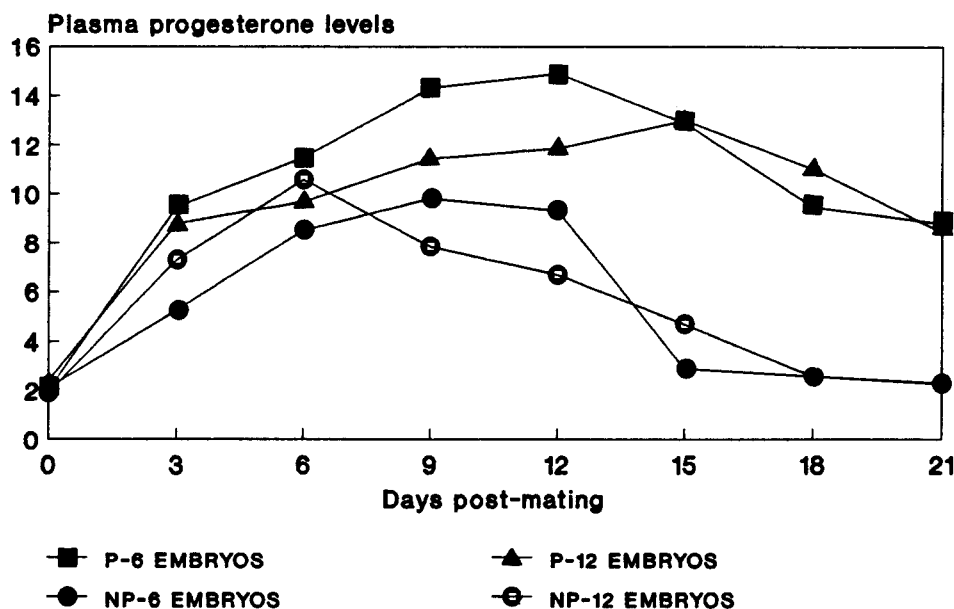
Litter size at birth was 3.7 ± 0.4 and 4.7 ± 0.5 respectively.

Plasma progesterone concentrations in recipient does are shown in Figure 1. They were higher in pregnant females (at least P<0.05) from Day 9 of pregnancy to the last blood sample recovered (Day 21), although the progesterone pattern shown by the non pregnant does indicates that the induction of ovulation was effective. No significant effect of the number of transferred embryos (6 vs 12) was detected. Progesterone levels rose from the induction of ovulation to a maximum on Day 12 in pregnant does (13.4 ± 4.2 ng/ml) and on Day 6 in the non pregnant ones (9.4 ± 2.5 ng/ml).

DISCUSSION

Mean ovulation rates recorded in the present study for the Spanish Giant breed were slightly lower to the ones obtained in nulliparous does of the same breed by FORCADA and ABECIA (1989) using entire males and by FORCADA *et al.* (unpublished results) using hCG injection, with 10.8 and 10.5 corpora lutea respectively. Only one doe did not ovulate (treated with GnRH). While endovenous doses of 10-25 IU of hCG induce ovulation whether does are receptive to the buck or not (HULOT *et al.*, 1988), the treatment with GnRH assures the ovulation in 95 % of the receptive does but only in 72 % of the non-receptive

ones (THEAU-CLEMENT *et al.*, 1990). The use of receptive animals is recommended to obtain embryos from donor does in the case of both hormones.

One of the aims of our study was to assure a reasonably good embryo recovery rate with anaesthetized animals compared to that obtained in the literature with slaughtered does. Percentages of embryos recovered for hCG and GnRH treated does were 86 % and 79 % respectively . These figures are higher than those obtained by CARNEY and FOOTE (1990) (55 %) using anaesthesia at 19h post–coitus and similar to those reported by FISHER and MEUSER-ODERKIRCHEN (1988) (85 %) and VICENTE and GARCIA (1991) (89 %) at 65h and 72h post–coitus respectively in slaughtered animals. Our results indicate the adequate embryo recovery rates obtained from Spanish Giant does with anaesthesia. In previous studies, the same donor doe was operated on up to three times without the appearance of appreciable adhesions of the reproductive tract. Therefore, the present technique could be useful for reduced parental populations, which is the case of the Spanish Giant breed.

VICENTE and GARCIA (1991) reported a higher number of embryos found in the uteri from donor does treated with hCG in relation to the untreated ones (21 vs 6 % respectively) when the embryo recovery was performed at 64–66h post–coitus. It seems possible

that the use of hCG to induce ovulation provokes an alteration in oviductal motility causing an accelerated transit of the embryos (BOURGADE and HALBERT, 1988). These findings have not been confirmed in the present study although the embryos were recovered later, 72h after coitus ; the proportion of embryos in the uteri from hCG treated does was reduced (5 %) and not statistically different from that obtained from the GnRH ones (2.3 %). Approximately 84h following induction of ovulation is the time when rabbit embryos normally leave the oviduct and enter the uterus (ADAMS, 1958 ; HODGSON and PAUERSTEIN, 1976).

Percentages of pregnant recipient does were similar to those reported by other authors in transferring embryos at the morula stage using laparotomy (BATTISTA et al., 1987 ; BOLET and THEAU-CLÉMENT, 1988 ; VICENTE and GARCIA, 1991). The proportion of youngs born from pregnant doe were 59 and 41 % for 6 or 12 transferred embryos respectively (P<0.05). TECHAKUMPHU et al. (1987) and BOLET and THEAU-CLÉMENT (1988) obtained success rates of 65 and 74 % transferring around 12 embryos, but they carried out their evaluation on the 17th and 14th day of gestation, respectively. When more embryos are transferred into the uteri of the recipients, losses are higher both during placentation and at the later stages (HUIQING et al., 1987). ADAMS (1960) showed that overcrowding results in embryonic mortality primarily during the post–implantation period. Our results indicate the good performance after transfer of a reduced number of embryos per recipient doe especially when the results are referred to the pregnant does, with an increased profit of the recovered embryos from ovulation or superovulation treatments.

Peripheral plasma progesterone levels recorded in recipient does which did not become pregnant were close to those reported in the literature for pseudopregnant animals (HARRINGTON and ROTHERMEL, 1977 ; CAILLOL et al., 1983 ; FORCADA and ABECIA, 1990), which suggests the efficacy of the hCG treatment to induce ovulation. No differences between pregnant and non pregnant does were found up to Day 9 after induction of ovulation, indicating the lack of a local effect of the corpora lutea on embryo survival in rabbits (BATTISTA et al., 1987). After Day 12 one role of the rabbit conceptus may be to modify ovarian responsiveness to PGF-2α, even though its presence is apparently not necessary for maintenance of the corpora lutea until maternal recognition of pregnancy on day 12 (MARCINKIEWICZ et al., 1992). In the present study, the number of transferred embryos did not modify plasma progesterone concentrations for pregnant recipient does, which are similar to those previously reported for pregnant rabbits by BROWNING et al. (1980) and STOUFFLET and CAILLOL (1988), who observed the highest mean

level around days 12–13 followed by a gradual decline between mid–pregnancy and parturition.

In conclusion, the results of the present study indicate the good embryo recovery rates obtained from donor does under anaesthesia, procedure which allows subsequent treatments on the same donor does. The higher percentage of youngs born recorded when a reduced number of embryos were transferred (6 vs 12 per recipient doe) diminishes the losses of embryos with a moderate or high genetic value.

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