EFFECT OF RECIPIENT GENOTYPE ON SURVIVAL RATE AT BIRTH OF FROZEN LOCAL RABBIT EMBRYOS

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SUMMARY

New Zeland White (NZW) and APRI rabbit strains were used as recipients to study the survival rate at birth of frozen APRI embryos as well as the effect of site of ovary on ovarian activity, ovulation and embryo recovery rates. A total of 18 APRI rabbits line were super-ovulated using PMSG and naturally mated with bucks of the same line. Embryos were collected 64–66 h post coitum and the normal one was examined morphologically before frozing in liquid nitrogen. After thawing, embryos (n=140) were transferred into the oviducts of recipient does of both APRI and NZW does (n=8 both).

Results indicated that no significant effect of the site of ovary on ovulation rate. The percentage of ovulation and recovery rates were higher insignificantly in left ovary by 7 and 11% than right ovary, respectively. Average number of collected APRI embryos was 12.39 ± 0.83 and recovery rate 76.1 ± 16.2/doe. Examination of ovarian structure indicated that numbers of bleeding follicles, large follicle, and small follicles were 6.22±1.98, 9.89 ±2.13 and 9.56 ±2.38, respectively. Genotype influenced the pregnant rate being 75% in APRI vs. 50% in NZW as well as on survival rate, which was about 45% in APRI and 31.9% in NZW.

Keyword: Rabbit, breed, frozen embryo, transfer and survival rate

INTRODUCTION

The cryopreservation of rabbit genetic resources could be achieved by both males and females germ plasm, through freezing semen and embryos, which are routinely used nowadays. Embryo vitrification permits the rapid cooling with no risk of ice crystal formation in liquid medium (Dobrinsky, 2001). Vitrification is a simple technique and more cheaper than embryo slow rate freezing, however, embryo exposure to high levels of cryoprotectant additives (CPAs) may be deteriorated after devitrification (Leoni *et al.*, 2003).

Several protocols have been developed for the vitrification of rabbit embryos with *in vivo* satisfaction results (Vicente *et al.*, 1999 and López-béjar and López-Gatius, 2000), with difference rate of after thawing viability (Ogawa and Tomoda, 1976 and Vicente and Garcia- Ximenez, 1993). Strain was reported to affect pregnancy rate of thawed embryos and the general shape of embryos (Ogawa and Tomoda, 1976).

Previous data indicated that about 71% of vitrified embryos had the ability to be developed to blastocyst stage after *in vitro* culture for 72 h, and 23.5% of embryos transferred to four recipients, developed to term (Papis *et al.*, 2005). The study of Vicente (2008) revealed that 50% of cryopreserved embryos could be survived after transferring, however such percentages are depends on several factors, e.g. stage of embryos, genotype of donors and recipients (Vicente, 2008). The aim of this work was to evaluate the effect of genotype of recipient on the *in vivo* development of vitrified embryos rabbits.

MATERIALS AND METHODS

All chemicals used in this study were purchased from Sigma (Madrid, Spain), unless otherwise indicated. Rabbit does of NZW and APRI strains were used in this study as recipients of APRI vitrified embryos. A total of 20 multiparous APRI rabbit does (2^{nd} and 3^{rd} parities) was mated to bucks from the same line. After 64–66 hours post-coitum, the donor does were slaughtered and the reproductive tract was removed and the embryos were recovered by flushing with recovery medium (phosphate-buffered saline supplemented with CaCl (DPBS, Sigma D5773), 0.002 g/mL bovine serum albumin (BSA, Sigma A3311), 100 IU/ml penicillin and 100 µg/ml streptomycin) (RM) at room temperature (20–25° C). After recovery, embryos were examined morphologically to detect normal ones (morulae with both intact and regular mucin coat and zona pellucida, and homogenous cell mass). Normal embryos from each donor doe were washed twice in fresh RM and kept at room temperature until use (10 to 15 min). Ovulation rate (number of luteinized follicles with ovulation stigmas), number of small, large and bleeding follicles and the number of normal and abnormal embryos were recorded as reproductive traits.

A total of 187 embryos at morula stage were vitrified. Vitrification was carried out in two steps at 20°C. In the first step, embryos were kept for two minutes in 0.4 ml of vitrification solution (VS), (50% RM + 25% ethylene glycol + 25% phosphate buffered saline) and RM (VS1) at volume base (1:1) in a plastic culture dish. In the second step, embryos

were kept in 0.6 ml of the VS 2 (20% RM + 80% VS) (vol. /vol.) in a plastic culture dish. The pH value of the media and osmolarity were adjusted at 7.3-7.4 and 280-300 mOsmol/kg, respectively before being filtered by 0.22-µm millipore filter.

Embryos were suspended in the final vitrification solution and then 8 - 10 embryos were loaded into 0.25-ml plastic straws (IMV, L'Aigle, and France) in 0.1 VS2 solutions. Straws were sealed with polyethylene powder, and then plunged directly into liquid nitrogen. Embryos were exposed to the final vitrification solution for a total of 1 min. The straws contained three fractions separated by air bubbles, the first and third fractions consisted of RM medium, while, the second fraction consisted of VS2 medium.

Devitrification was performed by immersing the second and third sections of the straws in a water bath at 20 °C for 10–15 seconds. The cryoprotective solution was removed from the embryos in a two-step dilution procedure at room temperature (20–25 °C). The embryos were released into a plastic Petri dish containing 1 ml of 0.33 M sucrose in DPBS medium. After two minutes, the embryos were washed twice in fresh DPBS medium and scored morphologically before transfer. Only embryos with a homogenous cell mass and an intact mucin coat and zona pellucida were transferred (n=140).

A total of 16 multiparous does (APRI n=8 and NZW n =8) does were used as recipients. Ovulation was induced in receptive does 60–64 h before transfer with an intramuscular dose of 75 IU Human Chrionic Gonadotropin (hCG, Pregnyl, Nile Pharm, Co. Egypt). The recipients were anesthetized by an injection of ketamine solution at the rate of 1.2 ml/kg body weight. Oviduct embryo transfer was performed unilaterally, where 6 to 14 normal embryos were transferred to each recipient doe. *In vivo* survival rate was assessed based on birth rates, the number of total offspring born and the number of live offspring.

Number of ovulation (CL), follicles and recovered embryo were compared between the ovarian sites per donor and two recipients using one-way analysis of variance and the recovery rates were compared using Chi-square test.

RESULTS AND DISCUSSION

Data in Table (1) shows that the ovarian site had no significant effect on the ovarian structure, ovulation and recovery rate of APRI rabbits does. Response of APRI rabbit line to super-ovulation was 90%.

The present results are in agreement with the findings of Ali (2010), who stated that site of ovary did not affect the average number of bleeding follicles and embryo recovery.

Total number of detected corpus lutetium in the present study (16.3, Table 1) is higher than that reported by El-Keraby *et al.* (1991). The authors found that number of corpora lutea of does treated with 0.2 or 0.4 ml GnRH ranged between 7.4 and 10.3 in different breeds. Also, with the findings of Gosalvez *et al.* (1994) reporting number of corpora lutea of 11.0 for rabbit injected with 100 IU PMSG. Meanwhile, it is less than that reported by Lee *et al.* (1991) stating that number of ovulation points averaged 19.2 /female under PMSG treatment. Using different types of hormonal administration to induce ovulation (superovulation) for the R and V line rabbits, Mehaisen *et al.* (2005) recorded a higher number of ovulation sites (15.3 and 15.9) as compared to 14.3 and 13.8, in the study of Viudes-de-Castro *et al.* (1995), and 13.5 and 13.2 in the study of Vicente *et al.* (2003) for R and V lines, respectively. These differences may be due to the effect of the superovulation treatment and breed that were used.

The response to superovulation treatment depends on the type of hormone (Mehaisen *et al.*, 2005) and rabbit breeds (Viudes-de-Castro *et al.*, 1995; Bolet *et al.*, 2000 and Vicente *et al.*, 2003).

Data in Table (2) shows that the proportion of normal embryos was 83.8 % and abnormal embryos was 16.2 % in APRI rabbit line.

The *in vivo* development viability of the thawed embryos was assessed with the parturition rate and the embryo survival rate in two breed rabbit recipient (Table 3).

A primary problem in the cryopreservation procedures was the proportion of intact embryos (homogeneous cell mass and zona pellucida and mucin coat without damage) after devitrification or Thawing; APRI embryos showed the percentage of intact embryos (transferable), 74.9% of 187 vitrified embryos and abnormal embryos 11.8 %, Table 2. The recipient does became pregnant 12 days after induce ovulation had been induced (75%) in APRI line than 50% in NZW rabbits. Doe from the vitrified and transfer failed to give birth was 25% (2/8 rabbits) in APRI line and 50% (4/8 rabbits) in NZW rabbits (Table 3). The mean number of alive-born by pregnant recipients was 5.37 ± 1.21 in APRI Vs 5.65± 1.21 in NZW, respectively (Table 3). No significant differences were observed between the generations of the APRI and NZW rabbits for the variables analyzed.

Data in Table (4) shows the mean number of embryos transferred and the survival rate at birth both overall and in pregnant recipient does.

When the survival rate at birth was analyzed for APRI recipient does, significant differences were found between the two strains being (45.1%) in APRI recipient does *vs* (31.9%) in NZW recipient does. The mean survival rate of pregnant recipient does was (62.9%) in NZW recipient does and (61.5%) in APRI recipient does.

Maurer and Beier (1976) have observed a higher percentage of survival in Dutch – Belted embryos

than in NZW embryos (20% *vs.* 7%), while higher percentage of embryos, irrespective of their genotype and whether they had been frozen, develop in NZW recipient does than in Dutch – Belted recipient does (46% *vs.* 26%). Differences in survival rate of freezing-thawing stresses have recently being observed in mouse strains (Schmidt *et al.*, 1985, 1987 and Pomp and Eisen 1990). Pomp and Eisen, (1990) have found an opposited influence on survival rate between maternal and embryo genotype.

Studies including rabbits (Maurer and Beier, 1976) and rats (Breuel *et al.*, 1993) both showed that PGF had an inhibitory effect on the development of embryos; however, caution was emphasized in drawing comparisons among the species due to differences in embryonic development. PGF seems to prevent the normal compaction process which leads to poorly developed embryos. Embryos exposed to PGF during early development become arrested at the morula stage of development. Embryos may be particularly sensitive to the embryotoxic effects of PGF (Scenna *et al.*, 2004).

In contrast, Putney *et al.* (1988) reported that the overall mean pregnancy rate was higher for dairy heifers compared to beef heifers, but there were no differences between dairy and beef cows. Also, there were no differences within beef cattle between Brahman and European breed-type recipients. However, it is widely accepted within the ET industry that conception rates are lower in recipients of *Bos indicus* background, whether pure or cross-bred, compared to *Bos taurus* recipients.

Table 1. Impact of site of ovary on the ovarian structure, ovulation and recovery rates in APRI does

Slte of ovary	Bleeding follicles	Large follicles	Small follicles	Corpora lutea	Embryo	Ovulation rate (%)	Recovery rate (%)
Right	2.78	5.28	4.61	8.22	5.83	43.21	70.9
	±0.87	±1.14	±1.16	±0.46	±0.78	±8.88	±7.8
Left	3.44	4.61	4.94	8.00	6.56	51.98	81.9
	±1.11	±0.99	±1.22	±0.76	±0.05	±8.43	±7.4
Total	6.22	9.89	9.56	16.28	12.39	47.34	76.10
	±1.98	±2.13	±2.38	±1.12	±0.83	±17.31	±16.2

Recovery rates =Embryos /CL*100
Ovulation rate= (Embryos /CL+LF)*100

Table 2. Recovery rate of flushing APRI rabbit embryo (normal and abnormal) and after cryopreservation by viterification method

Items	Recovery rates	
	N	%
Animal	18	
Corpora lutea	293	-
Total embryo	223	76.1
Normal	187	63.8
Abnormal	36	12.3
Vitrified		
Total vitrified embryo	187	-
Total embryo Recovery	162	86.7
Transferable (Normal)	140	74.9
Abnormal	22	11.8

Table 3. Pregnancy rate and bunnies per doe in recipient APRI and NZW does

Breed	Number of recipients	Pregnancy rate (%)	Bunnies/doe
APRI	8	75	5.37± 1.21
NZW	8	50	5.65 ±1.21

Table 4. Survival rate (%) of freezing APRI embryos in terms of recipient and embryonic genotype

Breed	Does	Transferred embryos	Survival rate (%)
APRI	8	8.9 ±0.7 (71)	45.1 (32)
NZW	8	8.6 ±0.5 (69)	31.9 (22)
		Pregnant recipient does	
APRI	6	8.71±0.6 (52)	61.5 (32)
NZW	4	8.75±0.7 (35)	62.9 (22)

Garcia *et al.* (2000) observed differences in the survival rate at birth between vitrified embryos belonging to lines V and R (43% *vs.* 22%, respectively). Probably due to the recipient genotype; in this respect, Vicente *et al.* (2003) transferred the vitrified embryos in a pool of recipient does of undefined genetic origin in a Uruguayan rabbit farm. This last aspect is another important factor in rabbits assessed by Vicente and Garcia-Ximénez (1993) and Joly *et al.* (1996).Obtain high fertility and survival rates, line V and line 1077 seemed to be better recipients than the lines which have NZW origin.

The hCG has been used to stimulate ovulation or to reinforce coital stimulation in rabbits. A non-receptive doe that ovulates in response to hCG treatment shows insufficiently developed oviducts and uterus and a lower probability of becoming pregnant (Moody and McNitt, 1988). Bolet and Theau-Clement (1988) obtained success rates of implanted embryos (65 and 74%, respectively), but they carried out their evaluations on the 17th and 14th days of gestation, respectively. The proportion of implanted embryos observed by Vicente and Garcia-Ximenez (1991) was 68%.

Besides the different times to the onset of oestrus recorded in different ruminant breeds, breed has been indicated as a major factor contributing to the variation recorded in the ovarian response to superovulation (Donaldson, 1984; Vivanco *et al.*, 1994 and Ammoun *et al.*, 2006). In cattle, sheep and goats the number of corpora lutea recorded and number of transferable embryos recovered following superovulation differ between breeds (Donaldson, 1984; Baril *et al.*, 1989; Goel and Agrawal, 2005 and Ammoun *et al.*, 2006). In goats for example, a higher number of embryos (average of 10.1) was recovered in Alpine does, compared to Angora does (Average of 7.5) (Baril *et al.*, 1989). In addition, a higher refractoriness was recorded in Alpine goats, compared to Nubian goats following superovulation (Nuti *et al.*, 1987). However, the breed effect has been associated with the different prolificacy of the breeds, where a high prolific breed has been reported to respond better to exogenous gonadotrophins (Bindon *et al.*, 1986). It has also been found that sheep selected for prolificacy tend to be more sensitive to gonadotrophin treatment (Kelly *et al.*, 1983 and Bindon *et al.*, 1986). In contrast, Picazo *et al.* (1996) failed to establish a clear breed difference in ovarian response in three sheep breeds superovulated with FSH.

Genotype has been recognized to have a major effect on ovarian follicular development. Even though gonadotrophin treatment increases the follicular development in all breeds, the numbers of ovarian follicles which are recruited to ovulate differ in the different breeds (Ammoun *et al.*, 2006).

CONCLUSION

The survival rate of freezing embryos of the APRI strain increased when they were transferred to recipient does of the APRI strain. In this case a pregnancy rate of 75% and a survival rate of 45.1% at birth were obtained. Survival rate was improved in APRI recipient does attaining 45.1% *vs.* 31.9% in NZW recipient does.

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تأثير سلالة الأم المستقبلة علي معدل بقاء أجنة الأرانب المحلية المجمدة حية عند الميلاد

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يهدف هذا البحث الي دراسة تأثير التركيب الجيني لأرانب النيوزيلاندي الأبيض والأبري علي معدل بقاء أجنة أرانب الأبرى المجمدة عند الميلاد وكذلك دراسة النشاط المبيضي ومعدل التبويض وعدد الأجنة المتحصل عليها من إناث أرانب الأبرى بعد حقنها بهرمون PMSG . تم تجميع أجنة الأرانب في الطور التوتى من 18 أم أبري متعددة الولادات بعد 64 إلي 66 ساعة من التلقيح وهذه الأمهات لقحت طبيعي من ذكور نفس النوع. تم تجميد أجنة الأرانب الطبيعية في النيتروجين السائل. تم نقل الأجنة بعد إسالتها إلي قناه المبيض (منطقة القمع) في سلالتين من الأمهات المستقبلة هما النيوزيلاندي الأبيض (8 أمهات) والأبرى (8 أمهات). وكانت النتائج المتحصل عليها كالتالي:

ارتفع معدل التبويض والأجنة المتحصل عليها من المبيضين الأيمن والأيسر للأمهات المانحة بمعدل 7 و11% علي الترتيب وكان الفرق بينهما غير معنوي. متوسط عدد الأجنة المتحصل عليها من أمهات الأبرى 12.39±0.83 جنين بينما كان متوسط الأجنة المتحصل عليها 70.1% لكل أم. من الشكل الظاهري للمبيض وجد 6.22 حويصلة نزفية و9.89 حويصلة كبيرة و9.56 حويصلة صغيرة. كانت نسبة الحمل بعد 12 يوم 75% في أمهات الأبرى المستقبلة بينما كانت 50% في أمهات النيوزيلاندي الأبيض المستقبلة. كان هناك ارتفاعاً معنويا في معدل الأجنة الحية عند الميلاد من أمهات الأبرى المستقبلة 45.1% مقابل 31.9% لأمهات النيوزيلاندي الأبيض المستقبلة.

يتضح من هذه الدراسة كفاءة أمهات الأبري كأمهات مانحة للأجنة لأمهات مستقبلة.