ZOOLOGICAL SCIENCE 7: 85–91 (1990)

Embryo Transfer and Pregnancy Rate in the Golden Hamster (*Mesocricetus auratus*)

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ABSTRACT—Two series of experiments were carried out to determine the reason for the low pregnancy rate after embryo transfer in the hamster. Two culture media, TC-199 and TALP plus 20% FCS, were tested for flushing and transfer of embryos. The 4- to 8-cell embryos were recovered from mated females (donors) and transferred to female recipients synchronized by the same hormonal regimen. The pregnancy rate after the use of TC-199 and TALP plus 20% FCS were similar, 36.4% and 39.1% respectively, and compared with control females (80.0%). Only two of eight pregnant females in the TC-199 group and three of nine pregnant females in the TALP group delivered live young. The second series of experiments was carried out on three groups of females. Those in the first group were subjected to embryo transfer (ten, 4- to 8-cell embryos into right uterine horn). Those in the second group were mated and received only medium in the right uterine horn. In the third group, also mated, sham injections were performed in the right uterine horn. All females were autopsied on Day 14 of gestation. The pregnancy rate in females of Groups I, II, and III were 50.0, 59.1, and 60.0% respectively, and the percent of pregnancies with at least one normal developed fetus from the right uterine horn of these three groups were 20.0, 46.2, and 58.3% respectively. In females of Groups II and III, the level of pregnancies in the right uterine horn were 30.8 and 17.7 percentage units less than in the left uterine horn. The number of normally developed fetuses in pregnant females of Group I was 24.4%, similar to that in the first experiment. The numbers of all recovered fetuses and of normally developed fetuses in the right uterine horn of females of Group II were significantly lower (25.8 and 19.5 respectively) than in the left uterine horn (74.2 and 56.6% respectively). A similar tendency was found in females of Group III. Of 174 recovered fetuses from both uteri, 30.5% were from the right uterine horn and 69.5% from the left uterine horn and the levels of normally developed fetuses were 14.9 and 55.2%, respectively.

These results shown that the main reason for a decreased pregnancy rate after embryo transfer in the hamster is due to a trauma of the endometrium of the uterus and medium introduced into uteri, which may induce secretion of prostaglandins.

INTRODUCTION

The technique of embryo transfer has been increasingly used in domestic animals to enhance genetical performance and, improve productivity [1–5]. In humans, this procedure is used in conjunction with *in vitro* fertilization to overcome infertility due to impaired tubal function [6–10]. Embryo transfer has been widely used in laboratory animals for fundamental studies of fertilization, blastomere separation, or nuclear transplantation. Primarily these types of experiments have been performed in mice [11–17]. Considerably fewer experiments on embryo transfer have been done in hamsters, a species which seems to be especially suitable for studies on reproduction because of their early maturity, stable cycle length, high prolificacy, and short gestation. Blaha [18] reported that 49.2% of 6- to 8-cell embryos from young hamsters developed to term when transferred into young recipients, but that only 8.3% of the embryos of the same developmental stage developed into fetuses when transferred into old

Accepted April 13, 1989
Received December 22, 1988

recipients. Orsini and Psychoyos [19] transferred hamster blastocysts into ovariectomized and progesterone treated females, and found that some of the embryos could develop into live fetuses (7–12 days of gestation). Sato and Yanagimachi [20] transferred 1- to 2-cell embryos into the oviduct or 4- to 8-cell embryos into the uterus (using TC-199 medium) and found that 50 to 100% of the females receiving 4- to 8-cell embryos did become pregnant while none of the females receiving 1- to 2-cell embryos did so. Ridha [21] transferred embryos at different stages of development (1-cell to 8-cell) into the oviduct or uterus of hamsters after induced and natural ovulation and had 53–62% implantation rates in superovulated females. He was not able to produce full term live young. Fan *et al* [22] reported that the culture medium had a strong influence on the success of the embryo transfer. Increased survival rates are obtained in hamster embryo culture with the use of tissue culture medium 199 (TC-199) or Tyrode's solution supplemented with fetal calf serum (FCS) or pregnant hamster serum (PHS). According to Bavister *et al.* [23], the modified Tyrode's solution designated TALP (tyrodes-albumin-lactate-pyruvate) is the most suitable for embryo culture and transfer in hamster.

The objective of the present experiments was to determine whether the type of medium or other factors influence the pregnancy outcome results when 4- to 12-cell embryos are transferred to the hamster uterus.

MATERIALS AND METHODS

Mature golden hamsters (*Mesocricetus auratus*) 8 to 15 weeks old were maintained on a 12 h light: 12 h dark cycle. The estrous of each female was determined according to Orsini [24]. The day of vaginal discharge was designated as Day 1 of the estrus cycle. Hamster females (prospective ovum donors and recipients) were superovulated with an i.p. injection of 30 I.U. of PMSG (Serotropin, Teizo Ltd, Tokyo) on Day 1 of estrus at 0900 h followed by an i.p. injection of 30 I.U. hCG (Sigma Chemical Co., St. Louis, MO) at 1400 h on Day 4 (77 hr after PMS). Females to be used as donors were mated with fertile males and female recipients with vasectomized males 7–8 hr after the hCG injection. On Day 4 of pregnancy the donor females were killed by cervical dislocation and then the uteri and oviducts were excised from the donors and, during the first series of experiments, were flushed with 0.8 ml of TC-199 or TALP with 20% FCS. Recovered embryos were rinsed once and stored no longer than 35 min at 37°C before transfer to the recipient. FCS was from GIBCO Co., (Grand Island, NY) and TALP was prepared by the method of Bavister *et al.* [23].

Recipient females were anesthetized with 0.12–0.15 ml sodium pentobarbital (60 mg/ml) i.p. and their uteri exposed through a dorsolateral incision. During the first exploratory series of experiments, eight to twenty 4- to 12-cell embryo were drawn into a micropipette with 20 μl of TC-199 or TALP and injected into the uterine lumen near the utero-tubal junction. Control animals were either a) mated to fertile males, or b) mated and underwent a sham embryo transfer wtih injection of 20 μl of medium only. Control animals received the same hormonal regime as the experimental females. All females were allowed to deliver term fetuses, or if no pregnancy was evident by 17 days after mating, the females were killed by cervical dislocation and the uteri were recovered for examination.

During the first series of experiments, it was found that after sham transfer (medium only) into the uterus no pregnancies resulted in the injected horn. Therefore, a second series of experiments were performed on females divided among three groups. The same hormonal regime as described above was used. The right uterine horn of females in Group I (embryo transfer group) received ten 4- to 8-cell embryos in 20 μl of TALP. In Group II females (medium only transfer group), the right uterine horn received 20 μl of TALP alone and in Group III females (sham injection group) the right uterine horn received only the tip of micropipette. On Day 14 after hCG and mating, the females were anesthetized, killed by cervical dislocation, and examined for pregnancy. The fetuses were counted and measured for crown-rump (C-R) length, weight, and stage of development.

Student's t-test was used for statistical evaluation.

RESULTS

The results of the first series of experiments demonstrated that the pregnancy rate was 36.4% (8/22) with the use of TC-199 and 39.1% (9/23) with TALP plus 20% FCS. A significantly higher pregnancy rate was found in naturally mated, control females (80.0%, 16/20) which received the same regimen for superovulation ($P<0.05$). Only two of eight pregnant females with TC-199 and three of nine pregnant females with TALP plus 20% FCS delivered live young. In control females, the delivery rate was 93.8% and was significantly higher than in experimental females ($P<0.05$). The percent of transferred embryos that developed to fetuses of the two groups was 54.3 (51/94) and 36.9% (55/149) respectively. The numbers of embryos transferred to each recipient varied from 8 to 20. As mentioned earlier, no implantations occurred in uterine horns of naturally mated females which received an injection of medium alone.

The results of the second series of experiments to determine whether the trauma of transfer had a role in the restriction of pregnancy rate are shown in Tables 1 and 2. Only two of ten pregnant Group I females (20.0%) were pregnant 14 days after embryo transfer. In the females with a medium only transfer (Group II) or a sham injection (Group III) the total pregnancy rate in the right

TABLE 1. Pregnancy rate after embryo transfer, medium only transfer, or sham injection in the right uterine horns of hamsters

			No. of pregnant females with recovered fetuses on Day 14 of gestation			
			Right uterine horn (treated)		Left uterine horn (untreated)	
Treatment	Females	Embryos in both uterine horns (%)	Total (%)	with viable fetuses on Day 14 (%)	Total (%)	With viable fetuses on Day 14 (%)
Group I (embryo transfer)	20 (recipients)	—	10 (50.0)	2/10 (20.0)	0	0
Group II (medium only transfer)	22 (mated)	13 (59.1)	9/13 (69.2)	6/13 (46.2)	13/13 (100.0)	11/13 (84.6)
Group III (sham injection)	20 (mated)	12 (60.0)	10/12 (83.3)	7/12 (58.3)	12/12 (100.0)	10/12 (83.3)

All females were sacrificed on Day 14 of gestation for examination of the fetuses.

TABLE 2. Number of recovered fetuses after embryo transfer, medium only transfer, and sham injection in the right uterine horns of hamsters

		No. of fetuses recovered from females on Day 14			
		In the right uterine horn		In the left uterine horn	
Treatment	In both uterine horns	Total (%)	With viable fetuses	Total (%)	With viable fetuses
Group I (embryo transfer)	44 from 10 pregnant females	44 (100.0)	11 (25.0)	—	—
Group II (medium only transfer)	159 from 13 pregnant females	41 (25.8)*	31 (19.5)*	118 (74.2)	90 (56.6)
Group III (sham injection)	174 from 12 pregnant females	53 (30.5)*	26 (14.9)*	121 (69.5)	96 (55.2)

* Significantly different from control levels ($p<0.05$).

TABLE 3. Crown-rump length and weights of viable hamster embryos on Day 14 of gestation

Treatment (right uterine horn)	Right uterine horn		Left uterine horn	
	Crown-rump length (mm)	Weight (mg)	Crown-rump length (mm)	Weight (mg)
Group I (embryo transfer)	14.3±2.8[1]	589±246	—	—
Group II (medium only transfer)	18.1±1.4	816±178	18.6±2.1	679±243
Group III (sham injection)	16.2±1.8	655±198	15.8±2.9	734±177

[1] Values expressed as mean±(S.D.)

TABLE 4. Ovarian weight and relationship of the number of fetuses to the number of corpora lutea on Day 14 of gestation

Treatment	Right uterine horn (treated) No. of fetuses in relation to no. of corpora lutea			Left uterine horn (control) No. of fetuses in relation to no. of corpora lutea		
	Ovarian weight (mg)	Total (%)	Viable fetuses (%)	Ovarian weight (mg)	Total (%)	Viable fetuses (%)
Group II (medium only transfer)	58.1±0.0	4.8/24.3 (19.4)*	3.8/24.8 (15.5)	62.5±0.0	10.4/28.0 (37.1)	9.6/28.0 (34.3)
Group III (sham injection)	41.3±0.0	4.1/22.0 (18.8)	2.7/22.0 (12.5)	61.5±0.0	11.4/24.0 (47.4)	9.7/24.0 (40.6)

Group I (embryo transfer) not evaluated.
* Significantly different from control levels ($p<0.05$).

uterine horn in comparison to the left was decreased by 30.8 and 16.8% respectively. The viable pregnancy rate (embroys judged capable of being delivered live two days later) was reduced by 38.4 and 25.0 percentage units in Groups II and III respectively.

Forty-four of the fetuses in 10 pregnant females were identified after embryo-transfer (Group I) but only 25.0% of these were classified as viable (Table 2). This result was similar to that observed in the first series of experiments.

The crown-rump length and weight of normally developed embryos at 14 days of gestation are shown on Table 3. No significant differences were found between groups.

Ovarian weight, the number of corpora lutea, and the relation of the number of fetuses to the number of corpora lutea are shown in Table 4. No statistically significant differences were found between groups for ovarian weight or numbers of corpora lutea as expected. In both Group II and III, the relationship between the number of all fetuses to the number of corpora lutea were, in the right side, 19.4 and 18.8% respectively and, in the left side, 37.1 and 47.4% respectively, a statistically significant difference reflecting the decreased embryo survival in the treated right side.

DISCUSSION

In the first series of experiments, attention was directed to two culture media, which according to Bavister *et al.* [23] play an important role in the culture of hamster embryos and their successful development after transfer to pseudopregnant females. Work reported by Fan *et al.* [22] has shown that the best results in embryo development were with media TC-199 or Tyrode's solution supplemented with 20–30% of PHS or FCS.

The present results confirm these findings. Pregnancy rates were lower in our experiments than those reported by Blaha [18] who reported 49.2% developed fetuses after transfer of 6- to 8-cell embryos into the uterus, and were also lower than those of Sato and Yanagimachi [20] who reported a 66% pregnancy rate with 48% live fetuses after

transfer of 4- to 8-cell embryos. Ridha [21] reported a 58–59% rate of implantation after unilateral transfer of ten 4- to 8-cell embryos per female. These values are similar to our pregnancy rate (50%) in the second series of experiments, but Ridha was not able to produce live offspring.

Significantly lower pregnancy rates and number of live fetuses were recovered after embryo transfer than in control females. This suggested that factors other than the type of medium, such as trauma to the uterus during the transfer procedure, may play an important role in the restriction of the implantation rate and further development of transferred embryos.

The results of the second series of experiments with sham transfer of medium or the introduction of only the tip of micropipette into the uterine horn show very clearly that these procedures adversely affect the pregnancy rate and the number of implantations.

The pregnancy rates in females with the sham transfer of medium (Group II) and in females with sham micropipette introduction (Group III) are similar- 59.1 and 60.0% respectively (Table 1), but about 20% lower than in control females in the first series of experiments. Comparing the number of pregnancies in the right (treated) horn and the left (untreated) uterine horn to the number of pregnant females, it can be seen that pregnancy rates in term pregnancies with normal and degenerated fetuses as well as those with at least one normal fetus were significantly lower in the treated uterine horn. Significant differences between the treated and untreated uterine horns are seen in the numbers of recovered fetuses on Day 14 of gestation (Table 2). The total number of fetuses (normal and degenerated) in the treated horns were less by about 48 percentage units in females with medium only transfer and by about 39 percentage units in females with sham injection transfer. Similar significant differences were found in the number of normally developed fetuses at Day 14. Thses results demonstrate that the main factor restricting the implantation and normal development of transferred embryos is trauma of the uterus.

The physiological mechanism of this trauma effect is not known. Pharriss *et al.* [25] noted that trauma of the endometrium causes release of prostaglandins which, according to Horton *et al.* [26] and Kirton *et al.* [27], stimulate uterine contractility in rabbits and primates, and can induce abortion [28]. Spilman *et al.* [29] studied the effects of two isomers of PGF_2 (19(R)–19(S)–OH) and found that they were considerably less effective than PGF_2 in stimulating motility of the rabbit and monkey reproductive tracts, and that there are different species sensitivities for prostaglandins. Gutknecht *et al.* [30] found that PGF_2 at a dose level of 2 mg/day over any consecutive three-day period from Day 4 after coitus were 100% effective in preventing or terminating pregnancies in the rat. The same author [31], reported that PGF_2 administered subcutaneously at a dose of 0.1 mg/day on Days 5 through 7 post-coitus in the hamster lowered both plasma and ovarian progesterone levels and terminated pregnancy in all animals. He also reported histological evidence of luteal degeneration on Days 6 and 7 post-coitus in treated animals, but that exogenous progesterone maintained pregnancy in PGF_2 treated females. According to Pharriss *et al.* [25] the hamster is the most sensitive species to prostaglandin-induced luteolysis of any examined animals. Thomas *et al.* [32], taking blood and tissue samples from hamsters during estrous cycle and for the first four days of pregnancy, found that maximum steroid and prostaglandin concentrations occurred around ovulation and after that declined to the lowest level on Days 3 and 4 of pregnancy. They reported that a close relationship exists between steroids and prostaglandins. These findings suggest that the trauma which probably releases prostaglandins during embryo transfer, is the main factor decreasing implantation and embryo development.

ACKNOWLEDGMENTS

These studies were supported by REED funds from the state of Michigan and grants from the National Institutes of Health. Appreciation is expressed to Mr. W. E. Roudebush for assistance in the procedures.

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