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Short communication

BLASTOCYSTS PRODUCTION AND COLLECTION IN ALBINO SYRIAN HAMSTER USING SUPEROVULATION AND INTRAUTERINE ARTIFICIAL INSEMINATION IN NON-BREEDING SEASON

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Summary

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In vivo blastocyst production and collection using superovulation and intrauterine insemination was established in albino Syrian hamsters. Twenty female albino hamsters were injected pregnant mare serum gonadotropin (PMSG, 25 IU) in non-breeding season and 48 h or 56 h later, 25 IU of human chorionic gonadotropin (hCG) were injected. Both groups were divided into two subgroups of natural mating and artificial insemination. The former group was mated with a fertile male (1 male for 2 females) after hCG injection and in the next morning, the hamsters with vaginal plug were regarded as pregnant. In the artificial insemination group, intrauterine artificial insemination of 1×10^8 sperms was done 12 h after hCG injection. Blastocysts were counted at 3.5 days after mating or insemination. However, 48 h and 56 h hCG and natural mating and 48 h hCG and artificial insemination were without blastocyst; however the method of 56 h hCG and artificial insemination produced of 15±5 (mean and standard deviation) blastocysts in each albino hamster in the winter.

Key words: artificial insemination, blastocyst, hamster, non-breeding season, superovulation

Some hamster strains including golden hamster, Brandt's hamster, and Romanian hamster are endangered (IUCN, 2012). To preserve vulnerable animals, assisted reproductive technologies can be used including collection and preservation of genome or cells such as fibroblast, semen, and embryos at different stages of development especially blastocysts (Mehrabani *et al*., 2014).

Blastocysts can be produced by natural mating or artificial insemination. Hamsters have a seasonal pattern of reproduction and cold weather stops routine estrous cycles in female golden hamsters (Simonneaux *et al*., 2012). Therefore, pro-

duction of blastocysts by superovulation and artificial insemination can be proposed. To increase more blastocysts in the hamster, pregnant mare's serum gonadotropin (PMSG) for ovarian stimulation and superovulation following human chorionic gonadotropin (hCG) for ovulation induction is applied (Sarsaifi *et al*., 2013). In hamsters, embryo production also has been induced using PMSG alone (Doetschman *et al*., 1988).

Apart from the type and number of hormones for superovulation, timing and type of insemination can affect the result of superovulation. Embryos have been produced with various injection interval timing of hormones (PMSG and hCG) in different rodents, such as 50 h interval in rats (Cornejo-Cortes *et al*., 2006), 48 h interval in mice (Ertzeid & Storeng, 1992) and 56 h interval in hamsters (Lee *et al*., 2005).

In many studies, embryos are obtained by natural mating in hamsters (Lee *et al*., 2005) nonetheless in another research embryos are produced by artificial insemination (Smith *et al*., 1987). The aim of the present study was establishment of *in vivo* blastocysts production and collection using superovulation and intrauterine insemination in albino Syrian hamsters during non-breeding season.

All procedures and treatments were performed according to the Animal Care Ethical Rules of Shiraz University of Medical Sciences, Shiraz, Iran. Twenty adult female albino hamsters (100±10 g) were selected in the diestrous phase using vaginal smears evaluation. PMSG (25 IU, Pregnecol, Bioniche Animal Health, Armidale, Australia) was intraperitoneally injected. The hamsters were divided into two groups which were intraperitoneally injected hCG (25 IU, LG Life Sciences, Jeonbuk-do, Korea) after 48 h (the first group) and 56 h (the second group). Each group was divided into two natural mating and artificial insemination subgroups (n=5) (Table 1).

Hamsters of the natural mating groups were mated with fertile males after hCG injection (1 male for 2 females) and in the next morning observation of the vaginal plug was regarded as pregnancy. In the artificial insemination groups, 12 h after hCG injection, intrauterine artificial insemination was done in both horns. In detail, for sperm collection, a young adult male albino hamster was euthanised by ketamine (50 mg/kg, Woerden, Holland), xylazine (5 mg/kg, Woerden, Holland) and cervical dislocation. Tails of epididymis of the hamster were isolated under sterile conditions and were chopped with

Table 1. Experimental design for evaluation of the effect of superovulation and intrauterine artificial insemination for blastocysts production and collection in albino Syrian hamsters in non-breeding season

Groups	Time (h)				
	0	48	56	60	68
G1 (n=5)	PMSG	hCG + NM			
G2 (n=5)	PMSG		hCG + NM		
G3 (n=5)	PMSG	hCG		AI	
G4 (n=5)	PMSG		hCG		AI

Abbreviations: PMSG, pregnant mare serum gonadotropin; hCG, human chorionic gonadotropin; NM, natural mating; AI, artificial insemination

a scalpel and were transferred into a 1.5 mL microtube containing 1 mL phosphate buffered saline solution (PBS, 37.5 °C) and were incubated in a dry incubator (Iran Khodsaz Co., Tehran, Iran) for 20 min. Then, sperm motility was examined on a 37.5 °C warm slide under a microscope. Sperm concentration was determined using hemocytometer counting. After determination of appropriate concentration, 1×10^8 sperm/mL was injected into each uterine horn using a 37.5 °C warm insulin syringe (Fig. 1A). For this purpose, female hamsters were anesthetised by ketamine and xylazine. Abdominal hairs were shaved and then were disinfected with povidone-iodine solution and draping was done. An incision about 1 cm was done in midline region of abdomen. Uterine horns were exposed using a forceps. Intrauterine injection of 0.2 mL sperm solution was performed using a 26 gauge needle inserted into an insulin syringe in a 30 degrees angle with uterine lumen in the cranial third of each horn (Fig. 1B). The uterine horns were returned to their place. Muscles and skin were sutured with 4-0 vicryl suture and 3-0 nylon suture, respectively. Finally, tetracycline antibiotic was sprayed on the skin and postoperative recovery was monitored.

After 3.5 days post-coitus or post-artificial insemination, uterine horns were flushed by PBS to collect blastocysts. In detail, each female hamster was euthanised by ketamine (50 mg/kg), xylazine (5 mg/kg) and cervical dislocation. Under class I laminar flow (Jal Tajhiz Labtech Co., Karaj, Iran), alcohol was sprayed on abdomen and hamsters were dissected. The uterus was cut from ovaries and vagina and was transferred to a plate containing PBS under sterile conditions. The uterine horns were filled with 37 °C PBS flushing medium (Fig. 2A) and then the cranial tips were cut using a scissors. Blastocysts were collected and counted (Fig. 2B) under a loop microscope (Optika, Italy).

Albino hamsters submitted to 48 h and 56 h hCG with natural mating and 48 h hCG with artificial insemination protocols were without blastocyst. The method including 56 h hCG and artificial insemination produced 15±5 (mean±SD) blastocysts in each albino hamster in winter.

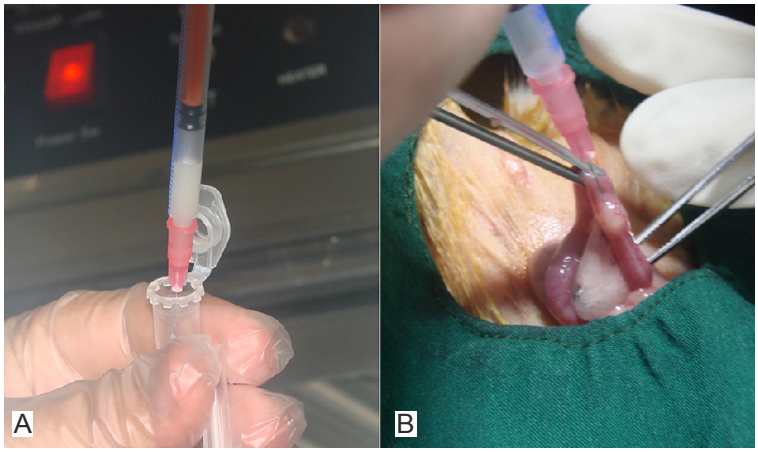

Fig. 1. A. Collection of counted albino hamster sperms from 37.5 °C incubated tail of epididymis in phosphate buffer saline; **B.** Intrauterine artificial insemination of female hamster.

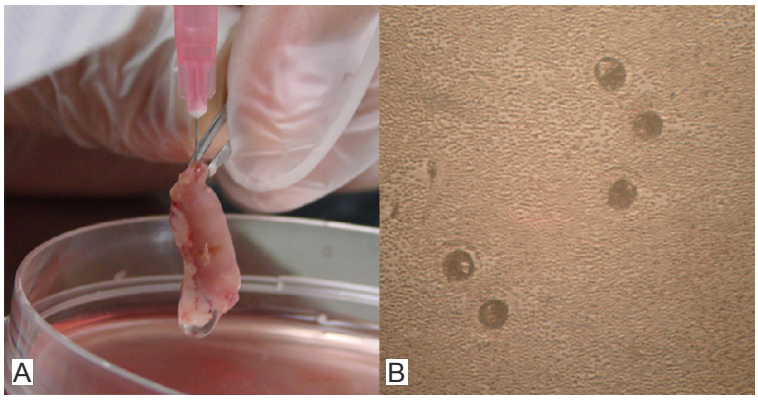

Fig. 2. A. Hamster uterine flushing by phosphate buffer saline; **B.** Collected hamster blastocysts.

Hamsters' superovulation with PMSG following hCG has been done in various studies. In a study, 409 blastocysts were produced in 31 hamsters (about 13 blastocysts per hamster) using PMSG without hCG (Doetschman *et al*., 1988). Consistent with our findings, 28 to 29 blastocysts yield per hamster with the same proportion of both hormones as used in the present study were obtained in breeding season (Lee *et al*., 2005). Superovulated hamsters (PMSG and hCG with a 56 h interval) had a rise in blastocysts number from 15 (control group) to 28–29 (Lee *et al*., 2005). Likewise, mouse superovulation (PMSG and hCG with a 48 h interval) increased blastocyst numbers from 33 (control group) to 52 (Ertzeid & Storeng, 1992). In addition, in Wistar rats, most embryo numbers were obtained by PMSG and hCG with 50 h interval (Cornejo-Cortes *et al*., 2006). Therefore, the application of PMSG and hCG at 56 h interval can induce superovulation in non-breeding season in albino hamster.

Intrauterine injection of 0.2 mL suspension containing 1×10^8 sperms in non-breeding season in superovulated albino hamsters produced blastocysts. The method by which artificial insemination is done varies as the sperm volume. Consistent with our findings, hamsters have been inseminated by the same operation with 1.7×10^8 sperms/mL in a 0.2 volume into each uterine horn (Smith *et al*., 1987). Rats were injected through surgical exposure into each uterus by 10×10^6 to 20×10^6 spermatozoa in a 0.1 mL sperm suspension volume (Orihuela *et al*., 1999). Therefore, intrauterine artificial insemination can be suggested for blastocyst production in hamster during non-breeding season.

In hamsters, breeding ceases during the winter months and gonadal regression occurs (Heldmaier & Steinlechner, 1981). In the laboratory, when hamsters were exposed to short winter-like non-breeding photoperiods, gonadal regression occurs and in both males and females, levels of offensive aggression were elevated (Jasnow *et al.,* 2000). Therefore, during non-breeding season inactivation of male and female gonads reduced the chance of success in reproduction even after superovulation and natural mating.

In conclusion, equal doses of PMSG and hCG (25 IU) applied at 56 h interval and intrauterine artificial insemination produced 15±5 blastocysts in each albino Syrian hamster during the non-breeding

season. Nevertheless, natural mating after PMSG and hCG (48 h or 56 h interval) and artificial insemination after PMSG and hCG (48 h interval) were without blastocyst during the non-breeding season. This study presented a suitable timing, rapid and practical method to produce and collect blastocysts using superovulation and intrauterine insemination in albino Syrian hamster in non-breeding season.

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