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ORIGINAL ARTICLE

Effect of Race and Hormonal Factors on Egg Production in Mice

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ABSTRACT

Production of large numbers of mouse oocytes is of great important for cell study and embryology. In this research, the effect of two important factors, namely mouse strain and the types of hormones affecting superovulation in mice have been studied. For this purpose, 20 mice from three strains, namely NMRI, BALB/C and C57BL6/J (20 mice per strain) underwent superovulation program using PMSG, HMG and GnRH hormones. The hormonal program for ovulation included injection of one of the three hormones to the mice of three races, followed by injection of HCG after 46 to 48 hours and then injection of HCG 15 to 20 hours later. By killing the mice, the oviduct was extracted, the ampulla was cut and oocytes were obtained. Among the three strains of mice, the NMRI strain provided the most and the best quality oocytes that were due to the fact that they were outbreed. Among the hormones, PMSG and HMG hormone were suitable for superovulation program. Since in research works, a lot of mouse oocytes are needed, outbreed mice like NMRI strain and PMSG hormone should be used for this purpose.

Keywords: *PMSG Hormone, HMG Hormone, GnRH Hormone, Mice and superovulation.*

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INTRODUCTION

Given that the timing of estrous cycle is not clearly visible in mice and they have short sexual cycle (5 days), production of large numbers of oocytes in laboratory requires using certain hormonal programs to induce ovulation in mice. For performing in vitro fertilization (IVF) and cloning the laboratory mouse, large numbers of oocytes should be produced.

The laboratory mice belong to class: Rodentia, subclass: Myomorpha, family: Muridae, genus: Mus and species: Mus Musculus. Their main characteristic is their long and sharp incisive teeth, and to inhibit their perennial growth, the mice need to chew things and nutrients constantly.

The body temperature of the mice is 36.5 °C to 38 °C, they breathe 100-200 times per minute, and their heart rate is 300-800 beats per minute.

Mice are poly-estrus animals and are able to reproduce all year round. The age of sexual maturation in mice is different and depends on strains, nutritional and environmental factors. However, it usually occurs 24-29 days after birth and the signs of puberty in female mice are vaginal opening and keratosis. Normally, estrous cycle in mice lasts for 4 to 5 days, and they start ovulating 8 to 11 hours after onset of estrus. Estrus typically occurs at night when the mice attack each other. Laboratory mice are normally capable of mating from 3 months of age, their gestation period is 18 to 21 days, and they give birth to 4-8 infants per delivery; regarding the infants, their eyelids are closed for 12 days, their bodies are hairless and their infancy takes 20 days.

Mating of mice occurs mostly during the night and consequently, the male semen is coagulated into the vagina of the female mouse and lasts for 16 to 24 hours, which is visible in the vaginal area and is called vaginal plaque. [1]

By planning the ovulation program in mice, this can be used for reproducing new generation of mice. In cloning programs of mice, purification of mouse strain is an important issue. Here, cloning program is done mostly in the first generation of a mouse strain and this can be achieved by setting out generation programs [2].

MATERIALS AND METHODS

Superovulation induction program

To achieve this goal, 20 female mice of NMRI strain, 20 mice of BALB/C strain and 20 mice of C57BL6/J strain were purchased from Pasteur Vaccine Institute, 20th Km, Tehran-Karaj Road, Iran on 2004.05.23 and were adapted for 21 days at a temperature of 20 °C to 28 °C, humidity of 40% to 50% and 12 hours light and 12 hours of darkness. During this period and later, the cages of the mice were cleaned 2 times a week, they were fed by plated nutrient, and carrots were given to them once a week. The mice were weighed after arrival, they were weighing 29 g when they were 4 to 6 weeks old, an ID card was prepared for each mouse. (Figure 1)

Figure 1: Location of mice, and foods used in this study

After this period, the mice of each strain were divided into 4 groups including 5 mice per group, and using various hormones, different hormonal programs were used as follows:

Group (1): 10 I.U. of Pregnant Mare Serum Gonadotropin (PMSG) hormone produced by Nasr Fariman Pharmaceutical Company (Registration No. 6010) was injected into mice intraperitoneally using insulin syringes and needle (No. 27); 46 to 48 hours later, 10 I.U. of Human Chorionic Gonadotropin (HCG) hormone manufactured by Intervet, Netherlands (23653) was injected intraperitoneally into mice. (Figure 2)

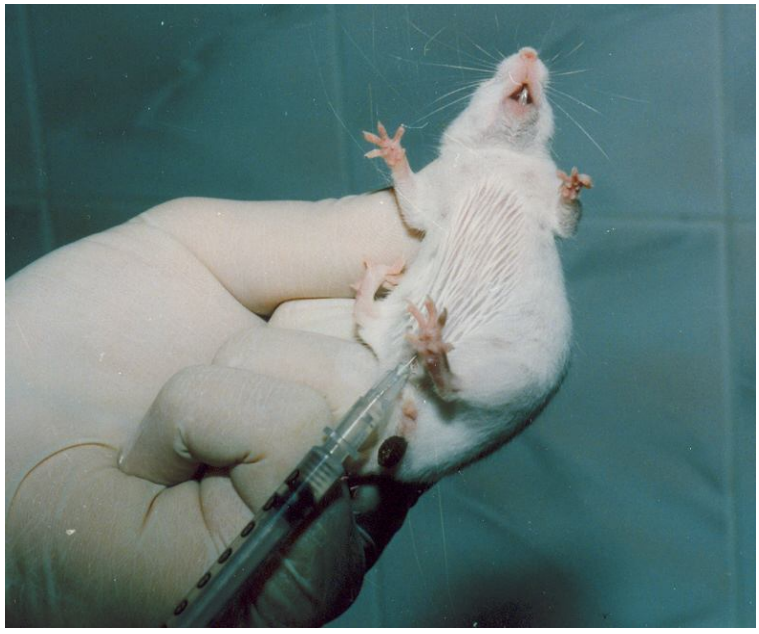

Figure 2: Intraperitoneal injection into mice

Group (2): They received 7.5 I.U. of Human Menopausen Gonadotropin (HMG) as an intraperitoneal injection, followed by injection of 10 I.U. of HCG hormone after 46-48 hours intraperitoneally.

Group (3): 10 I.U. of Gonadotropin-releasing Hormone (GnRH) produced by Aboureihan Pharmaceutical Company (Registration No. 78-9-H) branded as Vetarolin and injected intraperitoneally; followed by injection of 10 I.U. of HCG hormone after 46-48 hours intraperitoneally.

Group 4: 0.1 ml of normal saline injected intraperitoneally followed by the same amount of normal saline inject intraperitoneally after 46-48 hours.

Note: Prior to injection, the injection site was disinfected using alcohol 70%.
After hormone injection, in three periods of 15, 18 and 20 hours after injection of HCG, mice were anesthetized with ether-soaked cotton inside the desiccators, then with dislocation of cervical vertebrae they were killed under sterile conditions (alcohol 70%) and in the surgical tray, their skin, abdominal muscles and peritoneum were opened and by putting the intestines aside, their ovaries were exposed. (Figure 3).

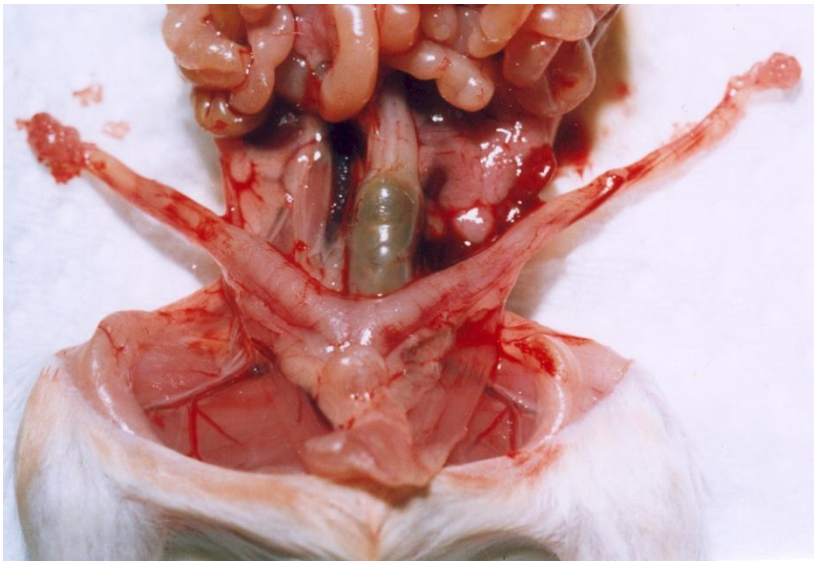

Figure 3: Uterus, ovaries and oviduct in mice

The forceps and surgical scissors were used to isolate fallopian tubes (oviduct), which were taken to Franklin Petri dishes (60*15mm, Lokes, USA) containing the previously prepared RPMI medium along with 5 mg Bovine Serum Albumin (BSA) in 1 ml of the culture medium.
Before transferring the fallopian tubes into the Petri dishes containing the RPMI medium, they were incubated for 5 min at 37 °C with 5% CO_2 so that oocytes would not be exposed to heat shock. The fallopian tubes were examined under a microscope equipped with loop and manufactured by Nikon Corporation. Oviducts of the mice with oocytes showed inflation in ampulla. With the help of insulin syringe needle (a sharp object) ampulla was cut, and in mice with ovulation, the oocytes along with cumulus cells around them were removed as a fused complex mass (oocyte cumulus) and were put in inside the Petri dishes containing the medium. The oocytes obtained were removed using a Pasteur pipette from Petri dish (with suction) and transferred to another Petri dish containing 10 drops of 50-100 ml of RPMI medium and containing 5 mg BSA per ml. These droplets were put gently at the bottom of the Petri dish using a 100 ml sampler, under the hood and beside a flame.

Isolating the cumulus cells surrounding the oocyte

As explained in the introduction, for performing IVF or removing oocyte for cloning the nucleus with the 2n number of chromosomes into it, the cumulus cells surrounding the oocyte should be removed. For this purpose, oocytes attached to the cumulus were placed in Petri dishes containing a drop of 50 ml of medium containing Bovine Testicular Hyaloronidase (0.1%-w/7). After 1 to 2 minutes, the cumulus cells surrounding the oocytes were starting to be separated and the Pasteur pipette was used to isolate oocytes immediately, which were moved three times to three adjacent drops containing no enzyme. Immediately, hyaluronidase enzyme was washed from the surface, since if hyaluronidase may remain more than a few minutes in the vicinity of the oocytes, zona pellucida of the oocytes will be broken down. After being washed, the oocytes were transferred to RPMI drops with 5 mg of BSA per ml. To prevent evaporation and changes in pH and microbial penetration into the drops containing the oocytes, their surface was covered by a sterile mineral oil manufactured by Sigma and incubated at 37 °C and 5% CO_2.

RESULTS

After application of hormones for ovulation of mice of group 1, 2 and 3, all mice ovulated at 15, 18, 20 hours after injection of HCG, but NMRI mice produced the most number of oocytes. (Table 1, 2 and 3)
Only mice in groups 1 and 2 which received Pregnant Mare Serum Gonadotropin (PMSG) and Human Menopausen Gonadotropin (HMG) produced oocytes and the mice in group 3 which received Gonadotropin-releasing Hormone (GnRH) produced no oocyte. Moreover, no oocyte was obtained from the control group.

The number of oocytes produced by the NMRI strain was higher than the oocytes from other strains and the obtained oocytes were of the highest degree morphologically. (Fig. 4)

Table 1: Total oocytes produced by NMRI strain with various hormones

	PMSG Hormone	HMG Hormone	GnRH Hormone	Control group
Mouse 1	35	30	0	0
Mouse 2	20	20	0	0
Mouse 3	25	28	0	0
Mouse 4	23	24	0	0
Mouse 5	30	23	0	0
Total	133	125	0	0

Table 2: Total oocytes produced by BALB/C strain with various hormones

	PMSG Hormone	HMG Hormone	GnRH Hormone	Control group
Mouse 1	10	10	0	0
Mouse 2	8	8	0	0
Mouse 3	7	9	0	0
Mouse 4	11	12	0	0
Mouse 5	13	10	0	0
Total	49	49	0	0

Table 3: Total oocytes produced by C57BLJ/6 strain with various hormones

	PMSG Hormone	HMG Hormone	GnRH Hormone	Control group
Mouse 1	10	10	0	0
Mouse 2	12	15	0	0
Mouse 3	15	13	0	0
Mouse 4	11	12	0	0
Mouse 5	13	10	0	0
Total	61	60	0	0

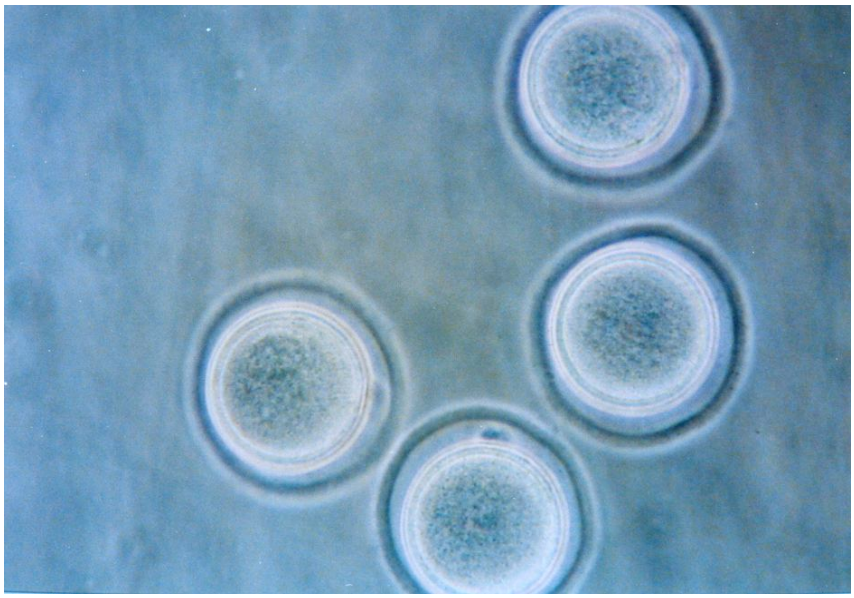

Figure 4: Oocytes derived from NMRI strain

DISCUSSION

Obtaining oocytes in the laboratory from mice is important for many purposes including cloning, and to have a high number of oocytes exact and regular hormonal programs must be implemented.

Wakayama et al. in their research program to clone mice obtained more than 2000 oocytes from female mice. The high number of oocytes was achieved using injection of PMSG hormone followed by HCG injection (46 to 48 hours later) and HCG injection (13 to 16 hours later) [2].

Also Dhanju et al, Kanter et al, Lesserre et al, and Kon SB et al in their research projects for hormonal induction of ovulation, injected 5 to 10 I.U. of PMSG followed by 5 to 10 I.U. of HCG hormone intraperitoneally 46 to 48 hours later, and obtained oocytes 13 to 18 hours later [3, 4, 5, 6]. Most of the scholars used the dose of 7.5 I.U. of hormones. We used the same programs and obtained similar conclusions and to ensure the efficaciousness of the hormones, we used the maximum dose of 10 I.U. Our proposed time for obtaining oocytes is 15 to 20 hours after HCG injection, which has no significant difference from the time used by the researchers.

In this study, HMG (Human Menoposal Gonadotropin) also provided similar results, however, because it is an expensive hormone so it is used less. None of the above researchers used GnRH hormone. Only Kanter et al in their study had used 10 I.U. of GnRH 24 hours before the injection of PMSG and found no significant difference [4].

Considering the above issues, for induction of ovulation in female mice, injection of 10 I.U. of PMSG intraperitoneally followed by injection of 10 I.U. of HCG hormone 46 to 48 hours later and taking oocytes from oviduct of the mice 15 to 20 hours later was confirmed as a successful procedure.

Among the three used strains in this study, NMRI strain that is an outbreed one produced the highest number of oocytes with the highest quality. The two other inbreed strains produced fewer oocytes. In inbreed strains due to fertility among siblings of a group, there is an increased risk of birth defects and the mice are weak in terms of sexuality and resistance to diseases. Consequently. For the research activities with mice that requires obtaining a large number of oocytes, outbreed mice are recommended.

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