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Induce Ovulation Using Different Doses Of Pmsg In Female Rat (*Ratus norvegicus*)

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ABSTRACT

Technique for induce ovulation has been applied, particularly when large numbers of embryos are required for embryo transfer. The purpose of the research was study of induce ovulation among group treated with different doses of PMSG in female rat.
All population of female rats were divided into three groups consisting of 10 animals each. Each animal were injected intra peritoneal at volume 0.1 cc. Doses of PMSG were 0 IU, 10 IU, and 20 IU, respectively, on the day minus two. Therefore, all animals were injected intra peritoneal on day zero. On day 4, all female rats were sacrifice and the uterus were taken out. A blunt 21 gauge needle was inserted through the cervic into the lumen of uteri. Then the Fallopian tubes were cut off. Embryo collection were recovered through flushing 1 cc PBS added with 20% FCS supplement.
The treated group were administered dose 10 and 20 IU PMSG tended to increased the number of eggs super ovulated, a number of embryos were found degenerated at the time of observation.
This research suggested that the doses of PMSG play an important role as a source of variation in induce ovulation.

Keywords: Induce Ovulation, PMSG, Morula, Blastula.

Introduction

Technique for induction of super ovulation in livestock industry has been applied, particularly when large numbers of embryos are required for embryo transfer. However, the use of large super ovulate doses of exogenous gonadotrophin has been reported to result in the reduction of fertility in the laboratory animals (Evans and Armstrong, 2004).

The yield of embryos may be highly variable and retarded or abnormal embryos are often recovered. In such circumstances, it is difficult to determine whether the primary defect occurred before or after the time of ovulation. Further studies - in which super ovulated rats were ovariectomized at different intervals after the time of mating, suggested that at least a large proportion of embryos during the first 36 hours after the time of mating were normal. The failure to develop to the blastocyst stage in super ovulated rats was as a result of abnormal ovarian hormone secretion like PMSG (Pregnant Mare Serum Gonadotrophin) after ovulation rather than abnormalities of oocytes (Miller and Armstrong, 2002; Poernomo, 2013).

The purpose of the research was study of induce ovulation among female rat treated with different doses of PMSG.

Methods

For the experiments, male and female LMR strain rats (*Ratus norvegicus*) aged 3 to 4 months with an average weight of 120 grams were used. The rats were allowed free access to food and water. Lighting was controlled and the animals were kept on artificial light cycle of 14 hours light and 10 hours darkness, instead of midnight defined as the midpoint of the darkness phase. According to Poernomo (1990) method, these normal lighting schedule would allow ovulation that normally begins shortly after midnight.

All population of female rats were divided equally into three groups consisting of 10 animals each. Each animal were injected intra peritoneal at volume 0.1 cc. Doses of PMSG (Intergonan® -Vemie) were 0 IU, 10 IU, and 20 IU, respectively, on the day minus two. Therefore, all animals were injected intra peritoneal on day zero through 10 IU Human Chorionic Gonadotrophin (Chorulon® - Interved). Day zero was the day when estrous phase occurred.

Each female rat was penned individually with male rat during the whole dark phase. Mated females were observed through the presence of vaginal plug in the following light phase. Unmated and male animals were not observed anymore.

On the day four, all mated females were sacrificed and the uterus was taken out. A blunt 21 gauge needle was inserted through the cervic into the lumen of uteri. Then the Fallopian tubes were cut off. According to the Monk (2007) technique, embryo collection were recovered through flushing 1 cc Phosphate Buffer Solution (PBS) added with 20% Fetal Calf Serum (FCS) supplement.

Developmental stage and morphology abnormalities of embryos were evaluated under phase-contrast microscope at 100 times magnified. The average of embryos collection were analyzed through analysis of variant.

Result and Discussion

Table 1 shows that the increasing dose of PMSG was used to super ovulated would result in increased number of mated rats and embryos collected. The treated group administered dose 20 IU PMSG tend to increased number of eggs super

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ovulated. On the similar way, the treated group administered dose 10 IU PMSG tend to increased too. However, there was no significantly difference between both 10 IU PMSG group and 20 IU PMSG according to the number of mated rats and average of embryos each rats on P>0.01.

The failure to collect embryos from super ovulated rats might be due to several reasons. According to the report of Donaldson (2003), overstimulation of ovaries could lead to a failure of the fimbriae to envelope the ovary at the time of ovulation because of a huge number of ovulation. Normally, each ovulation could tease *fimbriae* to get closer near ovulation cell through chemicaly ovotaxys. Unfortunately, there are huge number of ovulation cells that could cause failure of the *fimbriae* to recovered to the whole ovarium. On the other hand, some ovum cell might be lacking to catch up through *fimbriae* and plunge into the cavum abdomen. However, remain of the ovulation cell were collected from all of the treated rats, instead only two of ten were mates following embryos collection on the control rats.

Table 2 shows embryos were collected at different stage of development, whether all rats were mates at the same day. On the treated rats in both PMSG doses 10 IU and 20 IU, a number of embryos were found degenerated at the time of observation. The degenerated ovum was shown uncompacted or loss blastomer cells inside of the zona pellucida. These undoubtedly are caused by the high degree of fertilization failure. Some of oocytes resulted from early ovulation were probably caused by the direct LH-like effect of large doses of PMSG (Kostyk, *et al*., 2008).

The reason for the decline in fertilization rate in both treated rats 10 IU and 20 IU of PMSG has been associated with the increased of ovulation in relation to the time of mating. Table 2 shown that the increased dose of PMSG up to 20 IU affected wide range of developing embryos collected from 1-4 cell to late blastula. Embryos were collected on the stage 1-4 cell due to ovulate on the very late and failed to catch up the normal stage of development for fertilization. On the contrary, embryos were collected on the stage late blastula might be ovulate too early. The late blastula embryos were developed too mature on the tuba falopii, where early development of embryo was occurring. For the both too early or too late ovulation stage, probability of failure development was bigger than normal morula. These evidence on the previous research, where development of the embryos followed by vitrivication method (Bagchi, *et al*., 2008; Son and Tan, 2009).

Despite the small reduction in oocytes fertilization rate of the treated rats super ovulated with moderate doses of PMSG, the vast increased in number of fertilizable oocyte outweighed the disadvantage. However, normal fertilyze ability of super ovulated oocytes did not necessarily imply the normal embryo development. According to the Vander Hyden et al. (2006) the similar way of the embryo production occurred in the experiment of immature rats.

Conclusion

This research suggested that the dose of PMSG in the female rat play the important role as an induce ovulation in embryo collection.

References

1. Bagchi, A., Woods, E.J. and Critser,J.K. (2008). Cryopreservation and vitrification: recent advances in fertility preservation technologies. Expert Review Medical Devices. 5(3):359-370
2. Donaldson, L. E., (2003). The effect of prostaglandin $F_{2\alpha}$ treatment in superovulated cattle on estrus responses and embryo production. *Theriogenology*. 40:279-285
3. Evans, G. and Armstrong, D. T. (2004). Reduction in fertilization rate in vitro oocytes from immature rats induced to superovulate. *J. Reprod. Fert.* 90:131-135
4. Kostyk, S. K., Drophco, E. J., Moltz, H. and Swartwout, J. R. (1998). Oculation in immature rats in relation to the time and dose of injected human chorionic gonadotrophin or pregnant mare serum gonadotrophin. *Biol. Reprod.* 39:1102-1107
5. Miller, B. G., and Armstrong, D. T. (2001). Superovulatory doses of pregnant mare serum gonadotrophin caused delayed implantation and fertility in immature rats. *Biol. Reprod*. 45:253-360
6. Monk, M., (2007). *Mammalian Development*. IRL Press. Oxford-Washington D.C.
7. Nakahara, Y., Imanishi, S., Mitsumasu, K., Kanamoni, Y., Iwata, K., Watanabe, M., Kikawada, T., and Okuda, T. (2010). Cells from an anhydrobiotic chironomid survive almost complete desiccation. Cryobiology. 60:2, 138-146
8. Poernomo, B.S., (1990). Study of embryo manipulation on the rat. PhD. Disertation. Bogor Agricultural University. Bogor
9. Poernomo, B.S., (2013). Embryo collection toward different doses of PMSG in rat (*Rattus norvegicus*), Proceeding International Seminar; The Role of Veterinary Science to Support Millennium Development Goals, Surabaya
10. Son, W.Y., and Tan, S.L. (2009). Comparison between slow freezing and vitrification for human embryos. *Expert Review Medical Devices*. 6(1):1-7
11. VanderHyden, B. C., Rouleau, A., Walton, E. A., and Armstrong, D. T., (2006). Increased mortality during early embryonic development after in vitro fertilization of rats oocytes. *J. Reprod. Fert.* 77:401-408

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Appendixes

Table 1: Responses of treated rats to PMSG

PMSG (IU)	Number of Treated Rats	Number of Mated Rats	Number of Embryos Collected	Average of Embryos On Each Rats
0	10	2	12	6·00
10	10	6*	53	8.82*
20	10	8*	74	9.25*

*P>0.01, no significantly difference

Table 2: Stage development of collected embryos

PMSG (IU)	Number of Embryos Collected	Degenerated	1-4 Cells Stage	Morula	Early Blastula	Late Blastula
0	12	0	1	10	1	0
10	53	4	0	41	7	1
20	74	12	7	45	8	2

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