

**Original Article**

## **Effect of substitute milk on growth and breeding rates of hysterectomy derived guinea pigs neonates**

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### **ABSTRACT**

In this study, considering the normal ingredients in guinea pig's milk, by using cow milk, mixture of vitamins and minerals, a milk substitute was prepared and sterilized by autoclaving and filtration methods. In food analysis of produced milk, the important substances values, such as protein, fat and ash, were suitable. Milk substitute was fed to four groups of newborn's guinea pigs that were obtained by hysterectomy from out bred color Pirbright conventional parents, and maintained in the sterile environment inside laminar flow. The health, growth and reproductive parameters were evaluated during 6 months in four treated groups at the suckling age (milk substitute) and solid pellets feeding stage and these values were compared with four coequal's control groups. The results show, all young fed with substitute milk, reached to puberty at normal age (3-4 months old), The males, had the normal fecundity and the duration of pregnancy in all females were in the normal range ( $2^2$ - $2^5$  weeks), litter size (4-5) and birth weight (70-100g) were in the normal ranges. In this study the preparation of suitable substitute milk and also a part of the method and protocol for production of the specific pathogen free (SPF) animals were introduced and put into the practice.

**Keywords:** Substitute milk, Growth, Breeding, Hysterectomy, Guinea pig

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### **INTRODUCTION**

Use of laboratory animals in various fields, has increased dramatically, and millions of these animals for production of various biological products (vaccines, drugs) and also in several research such as genetics, biochemistry, microbiology, nutrition, immunology, oncology, infectious diseases, as a model. However, any animal can be used as a model, but rodents and small animals because of small and suitable size, high reproducibility, variety, easy access, requires less space

and convenience are the most used (Fox *et al* 2002, Terril & Clemons 1998, UFAW 2010). Since the process of making biological products is controlled on laboratory animals, therefore, the health of animal and be free from any kind of pathogenic agents is essential. Specific pathogen free (SPF) animals, have not microorganisms and macroorganisms causing diseases but have the other non-pathogenic organisms (Brown 1995, FELASA 2002, Clemons & Seeman 2011, NRC 1996, Walker & Poppleton 1967). For establishment of SPF animal core, providing specific circumstances and devices as resistive barrier, which protects against entering any contamination, are necessary. These

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animals are usually obtained by cesarean section or hysterectomy and immediately placed in a controlled environment. SPF animals diet should be free of pathogens. The suckling age is one of the most important step in producing the clean animals. Since newborns are derived by cesarean section from conventional parents, the milk substitute can be a good alternative to feed them. (ICLAS 1987, Eveleigh *et al* 1984, Owen & Porter 1967). The replacement like natural milk provides the nutritional requirements and should be free from pathogens by physical or chemical methods. (Lin *et al* 2013, Nicklas 2002, Scher *et al* 1969). Amounts of compounds in guinea pig milk including, protein, fat and ash, are respectively, %8.1, %3.9 and %0.8. In solid food the best values of important nutritional amounts including rates of protein, fat, fiber, calcium, phosphate and ascorbic acid in growth stage are: %18-20, %1.4-4, %15, %0.8, %4.4, %0.02 (Nelson *et al.* 1951, Terril and Clemons, 1998). Therefore the combination valve in substitute milk and solid food should be similar to requirements. The post suckling pups are fed with pathogen free pellets (sterilized by physical methods) that contributes to clean animals production (Lin *et al* 2013, Nicklas *et al* 2002, Scher *et al* 1969). There are some reports in production of substitute milk for various laboratory animals. Wostmann and Pleasants prepared the substitute milk for laboratory rabbit in 1959 and then used for SPF laboratory rabbit colony. Scher *et al.* (1969) have established the SPF laboratory rabbit colony, by hysterectomy. They got the newborn rabbits and fed them with artificial feed in the protective barrier breeding system. (Table-1) Rabbits were fed with substitute milk in suckling stage and pasteurized solid food thereafter.

## MATERIALS AND METHODS

**Preparation of substitute milk.** The substitute milk for guinea pig is prepared according to the rabbit milk replacement that was made by Wostmann and Pleasants (1959). In order to do so, the existing facilities were used and the

substitute milk was made either by modification of the milk values or addition of some materials as presented in the table 1 (Scher *et al* 1969). Compounds were sterilized either by the autoclave (compounds D, E and F) or by the use of 0.45 and 0.22 $\mu$ m filters (compounds B and C). Prepared solutions were placed in 4°C until use. At the consumption time, the solutions were poured into 300 ml water bottle, and the temperature was raised to 35-38 °C before use by the water bath. In consumption time, 5 ml solution B, 1 ml solution of C, D, E and 2 ml solution F were *added to solution A. In this stage, solution A was used as a milk substitute for newborn guinea pigs* (Scher *et al* 1969). To evaluate the nutritional value of milk, a sample was made and sent to the nutrition laboratory for analysis of various combinations particularly, rate of protein, fat, ash, dry matter and energy values, after sterilization and before use.

**Obtaining the newborns by hysterectomy.** In this applied research, the pregnant conventional Pirbright guinea pigs (out bred in the conventional lab. Animals facilities, Razi Institute, Karaj) with proven fertility were used 1-2 day prior to the parturition. Eight pregnant guinea pigs were selected, four animals were hysterectomized under laminar flow (Owen & Porter 1967) according to animal ethics guidance (ISIRI, 2008) and the other four kept in breeding room for normal parturition, as control group. All animals were Off feed, 12 hours prior to parturition or hysterectomy. For hysterectomy, the animal's ventral hairs were shaved and then according to ethical principles by mixing the two drugs, Ketamine and Xylazine, (44mg/kg and 5mg/kg) were anaesthetized via intramuscular injection into caudal thigh muscle (NCB, 2005, Fish *et al* 2008). After immersing the whole animal's body except the nose in a disinfectant solution (Deconex 1%, swiss) for 3-5 minutes, the animals were placed on sterile towels (autoclaved) and an incision was made in the midline, opened the abdominal cavity, and hysterectomy (removing the entire uterus) took

place. Pups were born after opening the uterus, and cutting the umbilical cord at 1-1.5 cm away from the abdominal surface without hitting ligation. The nostrils were cleaned and opened by sterile cotton swab and warm saline. After complete drying, they were transferred to the special cages (type 2 polycarbonate with a soft and autoclaved wood shavings bedding) under laminar flow. The temperature inside the laminar flow (class II, A, SCF 126, Beasat, Iran) was regulated on 37-40 °C.

**Feeding of newborns.** Four quaternary groups infants, fed every 8 hours, manually by an insulin syringe in the corner of the lip, was drained slowly. Desired amount of milk fed infants were considered. In order to accustom of infants to the drinking of substitute milk from water bottle, 100ml of substitute milk was poured and was placed on the cage manually. After 3 days, the manually feeding was finished and infants were fed by water bottle containing substitute milk until day 20. From day 10, the solid food (which was received pasteurization temperature) was placed at their disposal. All infants in the test and control group were monitored daily for their appearance, behavior, probability of occurrence of diseases. Yanabe *et al* (2001) have established the SPF guinea pig colony by the cesarean section and hysterectomy, the babies were kept in isolator and fed with the commercial substitute milk. The gamma radiation was used for milk sterilization. Infants were fed twice daily for 18 days, manually. Then they were fed by solid food that was sterilized with gamma radiation. The temperature inside the isolator on the first day was 36 °C and next 5 days was 31 °C and after it was set on 25 °C. After suckling stage, they were transferred to clean room that its temperature was 25-27 °C. The selected breeding systems were polygamous. (Yanabe *et al* 2001).

**Evaluation of reproductive parameters.** After weaning period, the experimental and control infants were separated and transferred to the conventional breeding colony. Their breeding system was polygamous (one male with three females). Their reproductive parameters such as, puberty age, fertility

rate, litter size and birth weight of newborns were evaluated in the experimental and control groups.

## RESULTS

### **Nutritional value of the substitute milk.**

Nutritional value of substitute milk is shown in table 2.

**Effect of substitute milk on the growth rates of infants.** Growth curve of guinea pigs fed substituted milk (experimental group) in comparison with control group in suckling stage is shown in figure 1.

**Reproductive parameters.** In all of experimental groups, the weight difference with control group were low in suckling stage and after feeding with solid food monitored over a period of 6 months and time of puberty were in the normal range. (3-4 months old). The males, had the normal fecundity and the duration of pregnancy in all females were in the normal range (2<sup>2</sup>-2<sup>5</sup> weeks), litter size (4-5) and birth weight were almost normal (70-100 g).

## DISCUSSION

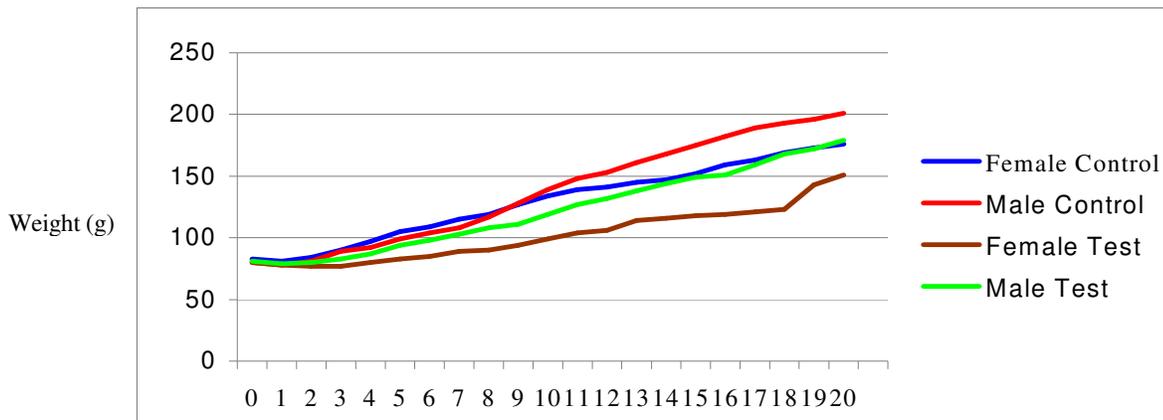
*Salmonellosis* is one of the most common food-borne bacterial diseases in the world (Fitzgerald *et al* 2003). Members of the genus *Salmonella* colonizes vertebrate hosts, with outcomes ranging from subclinical to systemic infection with high mortality. Animal infection has direct economic consequences, but asymptomatic carriage, leading to direct or indirect transmission to humans, maybe even more important (Songer *et al* 2005). Amounts of natural milk components for guinea pig includes protein, fat and ash, are respectively, %8.1, %3.9 and %0.8 (Nelson *et al* 1951). Comparison of the substitute milk components with natural one shows the produced milk constitutes the almost normal values and is suitable for replacement (Nelson *et al* 1951). The growth curves show that in all infants, the weight loss seen in early days after birth but then rised which is quite normal and due to changes in feeding and microenvironmental conditions. (Brown *et al* 1995) Compensation the weight loss in newborns of experimental group takes longer.

**Table 1.** Components and recipe of guinea pig substitute milk according to the model of Wostmann and Pleasants. (Scher et al. 1969).

Compound name	Ingredient	Amount	Recipe
A	Cow milk (Sterilized packaged, 3% Fat)	100ml	This combination reaches to 5ml volume by distilled water
B	Multi vitamin+Mineral	1gr	
C	Biotin	1 mg	This combination reaches to 100ml volume by distilled water
	Folic acid	10 mg	
	Vit C	100 mg	
D	KH <sub>2</sub> PO <sub>4</sub>	17.6 gr	This combination reaches to 100ml volume by distilled water
	Na <sub>2</sub> HPO <sub>4</sub> ,12H <sub>2</sub> O	45.4 gr	
	KI	40 mg	
	MgSO <sub>4</sub> ,7H <sub>2</sub> O	5.8 gr	
	MnCl <sub>2</sub> ,4H <sub>2</sub> O	0.7 gr	
	Ferric Ammonium Citrate	2.4 gr	
E	CuCl <sub>2</sub>	0.8 gr	This combination reaches to 100ml volume by distilled water
	ZnSO <sub>4</sub> ,7H <sub>2</sub> O	1.5 gr	
	COCl <sub>2</sub>	80 mg	
F	CaCO <sub>3</sub>	8 gr	This combination reaches to 100ml volume by distilled water

**Table 2.** Nutritional analysis of substitute milk

Nutritional Components	Amount /100 milk
Energy (Cal/g)	5472.6
Protein (%)	10.98
Fat (%)	2.8
Dry matter (%)	99.1
Ash (%)	1.9

**Figure 1.** Growth curve of substitute milk fed guinea pigs (experimental group) in comparison with control group in suckling stage.

The weight increments in suckling experimental groups were lower than the control suckling guinea pigs. (Owen & Porter 1967) Since the control infants were breastfed and non of the artificial foods or nutrition are as nutritious as mothers milk and in other hand milk

feeding in control groups take place without restraining stress that results to weight differences between the groups that seems logical. (Nelson *et al* 1951, Owen & Porter 1967) On the other, in the experimental group infants despite some differences in the weight gain,

they have ascending weight gain and without causing any symptoms suggestive of a particular disease such as hair loss, general weakness, wasting disease caused by the weakening of the immune and nutritional system and create mortalities have continued to grow. It should be noted that in experimental group infants, after suckling stage, the weight difference with control group were low and monitored over a period of 6 months, who were all in the normal range for age (3-4 months old) were adult. The mature males were fertile and had ability to produce pregnancy. The parturition time for all of pregnant females in experimental groups were in the normal range (2<sup>2</sup>-2<sup>5</sup> weeks) and the litter size and birth weight were also the almost normal (4-5 and 70-100 g). The results correspond with the results of other investigators. (Yanabe *et al* 2001, Owen & Porter, 1967) There are many reports in establishment of SPF animal colonies and use of substitute milk in the field. (Yanabe *et al* 2001, Lin *et al* 2004, Owen & Porter 1967). Yanabe *et al.* (1999) established the SPF rabbit colony with limited microflora. Yanabe *et al.* (2001) have established the SPF guinea pig colony by the cesarean section and hysterectomy, the babies were kept in isolator and fed with the commercial substitute milk (for dogs) plus vitamin mix (2%). Walker and Poppleton (1967) have produced, SPF colony of rats and mice. Lin *et al.* (2004) established the SPF New Zealand laboratory rabbit colony. They have obtained the newborn rabbits by caesarean section and were transferred into the isolator within 21-33 days and fed them by artificial milk. After suckling stage, they were transferred to the clean room. In their work at least 10 common pathogens in rabbits, removed and SPF animals were produced. Owen and Porter (1967) have produced SPF guinea pig colony. They also obtained infants through hysterectomy, and fed them with commercial substitute milk consisting vitamin supplements (420 mg/100ml) and minerals (2.25 g/100ml) and vit C (1 g/100ml). Pup's breast feeding was replaced by substitute milk syringe - feeding, two hours after birth, and repeated every 4hours for 10 days. From days 8-10, the solid food was placed at their

disposal. After 21 days, only solid meal they were given. Coates and O'Donoghue (1967) found that the young germ free rabbits, show a significant allergic reaction to cow's milk. The similar reactions were observed in guinea pigs as well (Campbell & Vier, 1978). In this research substitute milk was made and introduced that is suitable for guinea pig's normal growth and reproduction. This artificial milk is recommended to be used for SPF animals production.

### Ethics

I hereby declare all ethical standards have been respected in preparation of the submitted review article.

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### References

- Brown, C.L., Hahn, K.O. and Coleman, D.E. (1995). A Bibliography on the Culture and Maintenance of Specific Pathogen-Free Organisms, Center for Tropical and Subtropical Aquaculture Publication No. 116.
- Campbell, S.G. and Vier, F.J. (1978) The immunogenicity of milk from various species for the guinea-pig, *Clinical and Experimental Immunology* 33, 514-518.
- Clemons, D.J. and Seeman, J.L. (2011). The Laboratory Guinea Pig, Second Edition, CRC Press.
- Ethics of research involving animals, (2005) Published by Nuffield Council on Bioethics, 28 Bedford Square London, WC1B 3JS.
- Eveleigh, J.R., Taylor, W.T.C. and Cheeseman, R.F. (1984). The production of specific pathoden free rabbits, *Animal Technology* 35, 1-12.
- FELASA Working Group on Health Monitoring of Rodent and Rrabbit Colonies, (2002). Recommendations for the health monitoring of rodent and rabbit colonies in breeding and experimental units.
- Fish, R.R.E., Brown, M.J., Danneman, P.J. and Karas, A.Z. (2008). Anesthesia and Analgesia in Laboratory Animals, Second Edition, American College of Laboratory Animal Medicine.

- Fox, J.G., Anderson, L.C., Loew, F.M. and Quimby, F.W. (2002). *Laboratory Animal Medicine* 2<sup>nd</sup> edition, Academic Press.
- ICLAS Guidelines on the selection and formulation of diets for animals in biomedical research, (1987). A report from the nutrition working group convened by international council for laboratory animal science. Institute of biology, London, UK.
- Institute of standards and industrial research of Iran, (2008) Biological evaluation of medical devices- part 2: Animal Welfare Requirements . ISIRI, 7216-2, Available from www.isiri.org, (5 Mar 2008).
- Lin, Y.P., Chen, M.E., Chiul, H.M., Liang, C.F. and Hsu, T.L.(2013). Production of Primary Specific Pathogen Free Rabbits for Experimental Use AHRI report No.42, Branch Institute for Animal Drugs Inspection, Animal Health Research Institute 21 Mochang, Ciding Village, Chunan Township, Mioli County, Taiwan 35054.
- National Research Council, Institute for Laboratory Animal Research (1996). *Guide for the Care and Use of Laboratory Animals*, National Academy Press, Washington, DC.
- Nelson, W.L., Kaye, A. and Moore, M. (1951). Milking techniques and the composition of guinea pig milk, *The Journal of Nutrition* 44(4), 585-594.
- Nicklas, W., Baneux, P., Boot, R., Decelle, T., Deeny, A.A., Fumanelli, M., Ilgen-Wilcke, B. (2002). Recommendations for the health monitoring of rodent and rabbit colonies in breeding and experimental units. *Laboratory Animals* 36: 20-42.
- Owen, D.G. and Porter, G. (1967). The establishment of a colony of specific-pathogen-free guinea-pigs, *Laboratory Animals* 1:151-156.
- Scher, S., Collins, G.R. and Weisbroth, S.H. (1969). The Establishment of a Specific-Pathogen-Free Rabbit Breeding Colony, I. Producers for Establishment and Maintenance, *Laboratory Animal Care*, 19 (5), 610-616.
- Terril, L.A. and Clemons, D.J. (1998). *The Laboratory Guinea Pig*, CRC Press.
- UFAW Handbook on the Care and Management of Laboratory and Other Research Animals, (2010). 8th edition, Wiley-Blackwell.
- Wagner, J.E. and Manning, P.J (1976). *The Biology of the Guinea Pig*, Academic Press.
- Walker, A.I.T. and Poppleton, W.R.A. (1967). The Establishment of a Specific-Pathogen-Free (SPF) Rat and Mouse Breeding Unit, *Laboratory Animals* 1,1-5.
- Yanabe, M., Shibuya, M., Gonda, T., Asai, H., Tanaka, T., Narita, T., Sudo, K. Matsui, T. and Itoh, K. (1999). Production of Ex-Germfree Rabbits for Establishment of Specific Pathogen-Free (SPF) Colonies, *Experimental Animals* 48 (2), 79-86.
- Yanabe, M., Shibuya, M., Gonda, T., Asai, H., Tanaka, T., Sudo, K., Narita, T., Sudo, K. and Itoh, K. (1999). Establishment of Specific Pathogen-Free Rabbit Colonies with Limited-Flora Rabbits Associated with Conventional Rabbit Flora and Monitoring of their Cecal Flora, *Experimental Animals* 48 (2), 101-106.
- Yanabe, M., Shibuya, M., Gonda, T., Asai, H., Tanaka, T., Sudo, K., Narita, T., Matsui, T. and Itoh, K. (2001) Establishment of Specific Pathogen-Free Guinea-pig Colonies Using Limited-Flora Guinea-pigs Associated with Conventional Guinea-pig Flora and Monitoring of their Cecal Flora, *Experimental Animals* 50 (2), 105-113.