Ovarian response of immature Syrian female hamster treated with different doses of hMG

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Abstract

Human menopausal gonadotropin (hMG) is commonly used in human assisted reproduction. However, effects of hMG on follicular development remain controversial, due to its high LH- content. The present study aimed to elucidate the relationship between the dosage of injected hMG and the ovarian response of immature Syrian hamsters. Three groups of immature Syrian female hamster (n=10) were administrated intraperitoneally with 5, 10 or 15 IU of hMG, respectively. As a control group, 10 cyclic females were permitted coitus with males of proven fertility and allowed to deliver pups. Ovaries were collected at 2 d after hCG administration, and all follicles and corpora lutea were counted under a stereomicroscope. The hMG administration induced significant ($P< 0.05$) and distinct morphological changes in the ovaries, as it promoted the follicular development with a dose – dependent manner. The mean number of follicles was 29.2, 40.6 and 42.2, respectively, as compared to a mean of 9.6 follicles in the control group. However, a

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reduced ovulation rate was recorded as only 1.7, 10.4 and 16 corpora lutea were found in the ovaries. Overall, the present findings suggest that hMG, while suitable for inducing multiple follicular development in Syrian hamster, affected negatively on ovulation rates, thus, is largely ineffective for producing multiple embryos suitable for transfer.

Key Words: Syrian hamster, hMG, superovulation, follicular development

الاستجابة المبيضية لإناث الهامستر السوري الفتية المعاملة بجرعات مختلفة من hMG

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الملخص

يستخدم منشط المناسل الإياسي البشري (hMG) في حقل التناسل المساعد عند الإنسان، ولكن لازالت تأثيرات الـ hMG في التطور الجريبي متباينة نظراً لإرتفاع مستواه من الـ LH. هدفت الدراسة الحالية لتوضيح العلاقة بين جرعة الـ hMG المحقونة والاستجابة المبيضية عند الهامستر السوري. حُقنت 3 مجموعات من إناث الهامستر السوري الفتية (ن = 10) بـ 5 ، 10 أو 15 وحدة دولية من hMG ضمن الصِّفاق. سُمح في الشاهد لـ 10 إناث بالغة لتُلقح من ذكور مُخصِبَة، وأن تضع المواليد طبيعياً. جُمعت المبايض بعد يومين من حقن hCG وعُدّت الجريبات والأجسام الصفراء تحت المجسام. حرّض حقن hMG تغيرات شكلية معنوية ومميزة في المبايض، إذ حفّز التطور الجريبي بأسلوب مرتبط بالجرعة، وبلغ عدد الجريبات 29.2 و 40.6 و 42.2 على التتالي مقارنة بـ 9.6 جريبات عند الشاهد، ولكن سجل إنخفاض معدل الإباضة إذ لم يوجد على المبايض سوى 1.7 و 10.4 و 16 جسماً أصفراً على التتالي. توحي النتائج الحالية عموماً أنه على الرغم من ملاءمة hMG لتحريض تطور جريبات عديدة عند الهامستر السوري إلا أنه أثر سلبياً في معدلات الإباضة وبالتالي فهو غير فعّال لإنتاج أجنة متعددة ملائمة للنقل.

الكلمات المفتاحية: الهامستر السوري، hMG، إباضة فائقة، تطور جريبي.

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Introduction

In most mammalian species, superovulation may be induced successfully by either immunization against endogenous inhibin or administration of exogenous gonadotropins, such as follicle stimulating hormone (FSH), equine chorionic gonadotropin (eCG), and human menopausal gonadotropin (hMG) (Saleh, 2011). HMG has been derived from the urine of postmenopausal women and contains both follicle stimulating hormone (FSH) and luteinizing hormone (LH). The first preparation of human menopausal gonadotropin (hMG) was produced with equal amounts of FSH and LH (Maclin, 2000).

In the field of reproductive biology, the Syrian hamster (*Mesocricetus auratus*) is uniquely suited to studies in reproductive biology due to its invariant four-days estrous cycle, and the shortest gestation length (16 days) of any mammal (Lisk, 1985). Many attempts have been made to utilize the hamster in assisted reproductive technologies, such as male fertility (Tyler et al., 1981), superovulation (Sarsaifi et al., 2013), and *in vitro* fertilization (Parkening and Cisneros, 1988; Lane et al., 2000). Although many reports indicated successful induction of superovulation, no optimal superovultory treatment has been suggested.

To our knowledge, the effectiveness of hMG as a superovulatory hormone in Syrian hamster has not been studied. The main objective of this investigation was to study the endocrinological effects of ovarian stimulation of hamsters with hMG to determine whether this gonadotrophin can be used to add advantages to embryo transfer in farm animals.

Materials and methods Animals

Thirty immature hamster females, aged 4- 6 weeks, were randomly allocated to one of three equal treatment groups (10 females each). The females were group- housed (3 - 5 per cage) and had ad libitum access to water and standard dairy cattle diet. The lighting cycle was 14 h:10 h light : dark, respectively (1:00 on; 15:00 off). Females of the first group were superovulated with 5 IU, the second group with 10 IU, and the third

group with 15 IU of hMG. The total dose was divided into 2 doses. The first hMG dose was administered intraperitoneally at 13:30 on day 1, the second at 13:30 on day 2 and hCG was administered (75 IU , intraperitoneally) to all animals at 13:30 on day 3, after which, the females were caged singly with a fertile male overnight. The following morning at 7:00 a.m, all females were checked for coital plugs. 40 - 44 h later, the female were killed, the uteri with oviducts were exteriorized and all follicles and CLs were counted under a stereomicroscope.

Statistical analysis

Data were statistically analyzed using the program SAS® 9.2 according to the model fitted by the GLIMMIX procedure: $Yi = Xi\beta + Zi\gamma + \varepsilon i$ where, Yi is the response variable for the ith observation (total number of follicles and corpora lutea). The quantity Xi is a column vector of explanatory variables for observation i that is known from the experimental setting and is considered to be fixed (the treatment of superovulation). The vector of unknown coefficients β is estimated by a least squares fit to the data Y. The ε is assumed to be independent, normal random variables with zero mean and constant variance, and γ is an unknown vector of random-effects parameters (animal, weight) with known design matrix Z, and εi is an unknown random error vector whose elements are no longer required to be independent and homogenous. Differences between the mean values were tested for significance by t-test with the predicted difference PDIFF adjusted to Tukey (SAS 9.2; 2008).

Results

As shown in Table 1, the numbers of follicles increased significantly ($P<0.05$) in all treatment groups as compared to controls. However, the number of follicles per female did not differ ($P>0.05$) among hMG stimulated groups, although there was a trend for greater variability accompanied with increasing the dose. The number of corpora lutea did not differ significantly ($P > 0.05$) among the control and treatment groups, except for the first treatment group that received 5 IU of hMG. In this group, ovulation rate wasquite low (5.8%; $P < 0.05$).

Table (1) Mean number of follicles and corpora lutea (CLs) after induction of superovulation with hMG as compared to controls.

treatment	No. of follicles			No. of CLs	
	mean	SE		Mean	SE
Control	9.60 [a]	0.69		9.60 [ab]	0.69
hMG 5 IU	29.20 [b]	3.70		1.70 [a]	0.87
hMG 10 IU	40.56 [b]	7.34		10.44 [ab]	2.51
hMG 15 IU	42.22 [b]	8.33		16.00 [b]	4.39

Within column, values with different superscript differ ($p<0.05$).

Discussion

Regardless of the ovarian stimulation protocol, there will be females which do not respond appropriately. The results of the present study imply that some gonadotropins do not always reliably induce superovulation in Syrian hamster unlike other mammals (Lauria et al., 1982) and that the function of hMG in immature Syrian hamsters is not similar to that of eCG (يسوف والصالح، 2017). The findings indicate that, under the present conditions, hMG administration increased the number of developing follicles, however, the CL count was decreased, suggesting ovulation failure, or the follicles were not in full maturation to reach ovulation. The possible reason for this may be that the hMG is mainly effective at the follicle recruitment stage, but is ineffective to induce ovulation of all the selected follicles. It is well known that LH content in hMG preparations is quit high (1 : 1) (Maclin, 2000) which is not required for follicular maturation and steroidogenesis during the follicular phase, and may impaired final stages of follicular development. If this assumption is true, it seems appropriate to use high pure FSH preparations such as recombinant FSH. Ovulation failure after hMG

treatment may be due to the fact that hMG treatment might accelerate viable follicular atresia, which is recorded in other laboratory animals (Fujimori et al., 1989). Although the nature of the adverse effects of hMG on ovulation rate in Syrian hamster is unknown, these finding argues for the use of hMG in In-Vitro production of embryos instead.

Increasing follicular development with increasing the doses is reported previously after administration of other gonadotropins (Kanitz et al., 2002). However, it is not recommended to administer high doses of gonadotropins. It is well documented that the administering high doses of exogenous gonadotropins is often associated with several adverse effects, such as decreased rates of fertilization (Evans et al., 1981), abnormalities in embryo development and transport (Miller and Armstrong, 1981).

Conclusion

Overall, the present findings suggest that hMG, while suitable for inducing multiple follicular development, is largely ineffective for producing multiple embryos suitable for transfer. Unquestionably, the mechanism of follicular development and selection of antral follicles in Syrian hamster warrants further investigations to allow this attractive laboratory animal to become a more informative model.

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