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Human menopausal and pregnant mare serum gonadotrophins in murine superovulation regimens for transgenic applications

D.A. Brooke [a,*], N.M. Orsi [b], J.F.X. Ainscough [c], S.E. Holwell [a], A.F. Markham [a], P.L. Coletta [a]

[a] *Molecular Medicine – Level 9, Wellcome Trust Brenner Building, St. James's University Hospital, Beckett Street, Leeds LS9 7TF, UK*
[b] *Perinatal Research Group, Pathology & Tumour Biology – Level 4, Wellcome Trust Brenner Building, St. James's University Hospital, Beckett Street, Leeds LS9 7TF, UK*
[c] *Worsley Building, University of Leeds, Clarendon Way, Leeds LS5 9JT, UK*

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Abstract

Superovulation is a fundamental procedure for generating transgenic rodents. While various methods exist, zygote yield/quality remain suboptimal, making these techniques open to refinement. All require a follicle stimulating and a luteinising effect. The former can be induced by pregnant mare serum gonadotrophin (PMSG) or other compounds like human menopausal gonadotrophin (HMG). While HMG can double zygote yield compared to PMSG, no study has compared their effects on embryo quality. Embryo yield could also be increased with PMSG: timing administration at estrus may further improve follicular recruitment. This study compared: (i) the efficacy of HMG/PMSG for producing viable embryos for microinjection; and (ii) the effect of HMG/PMSG administration at estrus on embryo yield. Whitten effect-induced estrous C57/Bl6xCBA F_1 hybrid mice were superovulated as follows: PMSG (day 1; 5 IU intraperitoneally) or HMG (days 1 and 2; 1 IU intramuscularly); all received human chorionic gonadotrophin (hCG) on day 3 (5 IU, intraperitoneally). Zygotes were retrieved following mating, morphologically assessed and microinjected with innocuous ZhAT1R construct (expressing LacZ reporter and human angiotensin II type 1 receptor) before transfer to pseudopregnant recipients. Pups were tested for the transgene by Southern blot. Neither HMG nor PMSG proved superior in improving embryo yield, morphology and short-term post-microinjection survival. However, HMG group micromanipulated embryos all failed to establish a pregnancy/generate transgenic pups, unlike their PMSG counterparts. While HMG can be used for superovulation, it appears to increase embryo vulnerability to the long-term effects of microinjection. Furthermore, the embryo yields associated with HMG can be replicated by timing PMSG injection to coincide with Whitten effect-induced estrus.

Keywords: Mouse; Superovulation; Gonadotrophin; Transgenics; Pregnancy

1. Introduction

Recent advances in transgenics have opened up an extensive variety of new research opportunities involving embryo-based biotechnologies [1]. While transgenic rodents were not generated until some 15 years after the first published reports of microinjection methods [2], their use is now commonplace, and accounts for a third of experimental animals used yearly in the UK [3]. However, this rapid increase has also raised ethical concerns regarding the efficacy of existing of transgenic animal production protocols. While methodological improvements have focussed principally on parameters

* Corresponding author. Tel.: +44 113 3438422; fax: +44 113 3438702.
E-mail address: d.a.brooke@leeds.ac.uk (D.A. Brooke).

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such as animal weight, age, strain and timing of gonadotrophin injection [4], zygote production rates remain suboptimal and the creation of transgenic models still requires the use of numerous animals, superovulated females in particular. Another obstacle associated with such procedures rests with the quality of zygotes retrieved following superovulation: these must retain maximal viability in order to successfully endure both *in vitro* (e.g. oxygen tension) and micromanipulation-induced stresses (i.e. microinjection) [4,5].

Superovulation regimens typically require a follicle stimulating hormone (e.g. pregnant mare serum gonadotrophin, PMSG) and a luteinizing hormone (e.g. human chorionic gonadotrophin, hCG). PMSG is not the only commercially available preparation. In particular, human menopausal gonadotrophin (HMG) has been proposed to be a purer product than PMSG, whose use results in fewer inconsistencies in FSH:LH ratios, almost twice the zygote yield and, consequently, fewer animals/dissections required [6]. However, no detailed attempts have been made to compare the merit of these gonadotrophins on the quality of the resultant embryos for transgenic transfer to date. Furthermore, there may be other potential approaches for increasing embryo yield with the use of PMSG alone. In cattle, it has been reported that a transient rise in FSH levels promotes follicular development. While this yields both dominant and subordinate follicles, the latter can become dominant if the initial leading follicle is either removed, or if exogenous FSH is supplied [7]. In theory, this could be applied to laboratory rodents in order to maximise oocyte yield: in this way, FSH-like gonadotrophin administration at estrus may potentially allow the retrieval of oocytes from two cycles of follicular recruitment (both pre- and post-injection). In this respect, the choice of gonadotrophin may be critical. In mice, injection of PMSG at estrus is associated with the highest percentage of oocytes with a normal metaphase II plate chromosome distribution; it also results in the lowest percentage of denuded oocytes with aberrant morphology, which typically exhibit poor fertilization, decreased maturation promoting factor and mitogen-activated protein kinase levels [8]. Therefore, it is tempting to speculate that the reportedly lower efficacy of PMSG compared to HMG in follicle recruitment and oocyte production could be compensated for by injection at estrus, thereby also resulting in higher quality ova.

Therefore, this study aimed: (i) to compare the efficacy of PMSG and HMG as follicle stimulating agents in the production of viable microinjected embryos, and (ii) to assess the effect of timing of PMSG and HMG administration at estrus with respect to embryo yield.

2. Materials and methods

2.1. Superovulation and mating

C57/Bl6xCBA F_1 hybrid (CBA sire; C57/Bl6 dam) female mice were group housed (10 per cage) and had *ad libitum* access to water and Standard Expanded Beekay diet (B&K, Grimston, Aldborough, UK). These hybrids are used routinely in our unit for these purposes, and have the benefit of high fecundity/hybrid vigour. The lighting cycle was 14 h:10 h light:dark, respectively (5:30 on; 19:30 off). Humidity was maintained at 55–65% and the temperature at $21.5 \pm 1\,^{\circ}C$. Titration of PMSG and HMG was performed prior to the study in order to optimise superovulation regimens to the F_1 mice in order to remove dose-dependent artefacts in the observations. Both gonadotrophins worked best at the following concentrations: 5 and 1 IU for PMSG (intraperitoneal) (Dunlop Veterinary Supplies, Dumfries, UK) and HMG (intramuscular) (OriGene Technologies Inc., Rockville, MD 20850, USA), respectively (data not shown). The different routes of administration were chosen based on manufacturer's recommendations.

Females were superovulated between the ages of 6–13 weeks; we anticipated that this age range was suitable and would have minimal impact on oocyte/embryo quality based on previous reports [9]. Estrous cycle synchronisation was achieved through the Whitten effect on day −2 [10], and estrus was confirmed on the basis of vaginal cytology, as previously described [11]. PMSG or HMG were administered at 11:30 a.m. on day 1, as described above. A second HMG injection was performed at 11:30 a.m. on day 2 in the latter group, following manufacturer's recommendation, due to the labile nature of the product. hCG (Chorulon, Dunlop Veterinary Supplies) was administered (5 IU, intraperitoneally) to all animals at 11:00 a.m. on day 3, after which the females were caged singly with an F1 hybrid stud male overnight. Females on different regimens were allocated to stud males on alternate weeks to obviate any potential male-linked confounding variables. Females were checked for coital plugs the following morning at 7:00 a.m. This procedure was repeated in females ($n = 4$ per group per week) of the same age over a period of 4 weeks (i.e. $n = 32$ in total).