Arab Gulf J. Scient. Res., **11** (2), pp. 253-257 (1993)

Mouse Strain Variations to Superovulation by Human Post Menopausal Gonadotrophins and Human Chorionic Gonadotrophins

Ahmed R. Al-Himaidi

Zoology Department, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia

ABSTRACT. Two commercially available gonadotrophic hormones: human post menopausal gonadotrophins (Pergonal, Serona, Italy) and human chorionic gonadotrophins (Profasi, Serona, Italy) have been used to test their superovulation capcacity in females of three inbred mouse strains (Balb/c, CBA and C57BL/6). Females of the three strains have responded differently to the hormones and to the dosage regime used. The best superovulation response was by Balb/c females to 10 IU of the hormones. Superovulation was also stimulated in CBA females by 10 IU of the hormones. The response has declined in females of both strains when the doses of the hormones were increased to 15 IU. However, superovulation in C57BL/6 females was only stimulated by the highest doses (15 IU) of the hormones used.

The study of superovulation and *in vitro* fertilization (IVF) has advanced greatly in recent years. The induction of ovulation in laboratory rodents has become an important adjunct to IVF and has been achieved mainly through the follicle stimulating hormone (FSH) in the form of pregnant mare serum gonadotrophins (PMSG) followed by the injection of the human chorionic gonadotrophins (HCG) (McLaren 1967). However, the mean numbers of ovulations in mice were observed to be affected by several factors including the dose and combinations of the hormones used (Wilson and Zarrow 1962, Baukloh *et al.* 1982 and Edirisinghe *et al.* 1986), sexual maturity and the genetic constitution of the treated females (McLaren 1967, Gates 1971, Ackerman *et al.* 1983 and Ibrahim 1987) and the weight of the females used (Lang and Lamond 1966).

In the present study, two locally available commercial gonadotrophins (Pergonal and

Profasi, Serona, Italy) have been used to investigate their efficacy in inducing superovulation in various inbred strains of mice.

Materials and Methods

Mice

Three inbred strains of mice, Balb/c, CBA and C57BL/6 were obtained from the College of Pharmacy, King Saud University, from King Khalid University Hospital, College of Medicine, King Saud University and from King Faisal Specialist Hospital, Riyadh. They were kept in our laboratory under controlled conditions of 14-hr light and 10-hr dark cycle with an ambient temperature of 25 ± 2°C. Food and Water were provided *ad lib*.

Hormone injections and ova collection

The contents of each ampule (75 IU) of Pergonal or human post menopausal gonadotrophins (HMG) were dissolved in 1 ml of sterile normal saline, while the contents of that of Profasi (500 IU) or HCG were dissolved in 10 ml of sterile normal saline. Both hormone preparations were then injected intraperitoneally (ip) at the rate of 5, 10, and 15 IU, respectively into three groups of 10, 6-8 weeks old females of each mouse strain. The HCG was injected 48 hr following the inoculation of HMG. Then 10-12 hr following HCG treatment, the females were killed by cervical dislocation, the oviducts were removed to petri dishes containing normal saline and the ova were removed and counted under a stereoscopic microscope.

The results were statistically analysed using Student 't' test.

Results

A total of 1500 ova were collected from all females, 680 from Balb/c with a mean of 22.6 ova per female, 451 from CBA with a mean of 15.0 ova per female and 369 from C57BL/6 with a mean of 12.3 ova per female (Table 1).

Table 1. Variations in number of ova produced by females of three inbred strains of mice superovulated by human menopausal gonadotrophic and human chorionic gonadotrophic hormones

Mouse strain	No. used	Mean body weight (g)	Total No. of ova collected	Mean No. of ova per female
Balb/c	30	25.3	680	22.6
CBA	30	23.5	451	15.0
C57BL/6	30	21.1	369	12.3
Totals	90	–	1500	16.6

Strain variations

All females of the three mouse strains used showed an increase in ovulation rate with the increase in the hormone doses used from 5 to 10 IU. However, the number of ova collected from Balb/c and CBA females declined with the increase of the hormone doses from 10 to 15 IU. However, superovulation in C57BL/6 females only started at 15 IU of the hormones. The increase in the number of ova produced in response to the increase in the doses of the hormones from 5 to 10 IU was significant in Balb/c females, while that in CBA females was not statistically significant (Table 2).

Table 2. Variations in superovulation response by females of three inbred mouse strains to variations in the dose of human menopausal gonadotrophins and chorionic gonadotrophins

Mouse strain	No. used	Mean No. of ova collected at each hormonal dose		
		5 IU	10 IU	15 IU
Balb/c	10	16.0	27.5*	24.5
CBA	10	11.9	18.2	15.0
C57BL/6	10	8.9	9.9	18.1*

* increase is significant at $P<0.01$

Discussion

Superovulation with gonadotrophic hormones is of scientific interest. Moreover, hormones of follicle stimulating activity extracted from human pituitary glands and from human post menopausal urine have been successfully used in the treatment of infertility in women (Crooke *et al.* 1964, Gemzell 1965, and Gemzell and Roos 1966). However, superovulation experiments with such hormones have witnessed great variations in results in accordance with the strain of the mouse used (Ackerman *et al.* 1983, and Ibrahim 1987). Similar to the observations of Ibrahim (1987), Balb/c females have shown the best superovulation response to the hormones used. However, variations in the number of ova produced per dose per mouse strain have been observed in the present study. Moreover, Balb/c females has significantly shown the best superovulation response to the hormones used at the rates of 10 IU, while the C57BL/6 females shown such a significant superovulation response to the hormones used only at the dose rates of 15 IU. On the other hand, the superovulation response of the CBA females to the hormones used was higher at the dose rates of 10 IU, but the increase was not statistically significant. Compared to the previous observations (Ackerman *et al.* 1983, and Ibrahim 1987), the present results of superovulation observed in Balb/c females at 10 IU of HMG and of HCG is the best ever recorded.

The variations amongst mouse strains in response to the hormones used could be due to the genetic background of each strain. While the variations in response of the three mouse strains to the doses of the hormones used, could be due to the particular critical requirements of each strain to the hormones used (McLaren 1967). When that particular critical requirements of both of Balb/c and CBA females to the hormones used have

exceeded (the increase in the dose rates of both hormones from 10 to 15 IU), disturbances in the system of hormonal response control of both mouse strains might have taken place resulting in reduced ovulation. Moreover, variations in response to various levels of gonadotrophins or of steroids in general by the ovary or pituitary gland between different strains of mice (Durrant *et al.* 1980), might also account for the variations in induced ovulation rates observed in the three strains of mice used.

Acknowledgements

I am grateful to Prof. Dr. Hussein S. Hussein for his help in the preparation of the manuscript. Thanks are also due to my colleague Saleh Al-Shamrany for help during the work.

References

Ackerman, S.B., Swanson, R.J., Adams, P.J. and **Wortham, J.W.E., Jr.** (1983) Comparison of strains and culture media used for mouse *in vitro* fertilization. *Gamete Res.* **7:** 103-109.

Baukloh, A.E., Mettler, L., Seki, M. and **Mass, D.** (1982) *In vitro* fertilization of the mouse. *in:* **Hafez, E.S.E** and **Semm, K.** (eds) *In Vitro Fertilization and Embryo Transfer*, Alan Liss, Inc., New York, pp. 89-95.

Crooke, A.C., Butt, W.R., Palmer, R.F., Bertrand, P.V., Carrington, S.P., Edwards, R.L. and **Anson, C.J.** (1964) Clinical trial of human gonadotrophins. II. The effect of pituitary and urinary follicle stimulating hormone and chorionic gonadotrophins on patients with idiopathic secondary amenorrhoea. *J. Obst. Gynaec.* **71:** 571-585.

Durrant, B.S., Eisen, E.J. and **Ulberg, L.C.** (1980) Ovulation rate, embryo survival and ovarian sensitivity to gonadotrophins in mice selected for litter size and body weight. *J. Reprod. Fertil.* **59:** 329-339.

Edirisinghe, W.R., Law, H.Y., Ng, S.C., Chia, C.M. and **Ratnam, S.S.** (1986) Superovulation of mice with human menopausal gonadotrophins or pure follicle stimulation hormone in combination with human chorionic gonadotrophins and the effect of aging on *in vitro* fertilization. *J. IVF and EM.* **3:** 314-318.

Gates, A.H. (1971) Maximizing yield and developmental uniformity of eggs. *In:* **Daniel, J.C., Jr.** (ed), *Methods in Mammalian Embryology*, California, U.S.A., pp. 64-75.

Gemzell, C. (1965) Induction of ovulation with human gonadotrophins. *Rec. Prog. Horm.* **22:** 170-198.

Gemzell, C. and **Roos, P.** (1966) Pregnancies following trearment with human gonadotropins with special reference to the problem of multiple births. *Amer. J. Obstet. Gynec.* **94:** 494-496.

Ibrahim, S.A.M. (1987) *Differences Among Strains of Mice in Gamete Characteristics.* Ph. D. Dissertation, Colorado State University, Fort Collins, Colorado, U.S.A., (unpublished). 297 p.

Lang, D.R. and **Lamond, D.R.** (1966) Some factors affecting the response of the immature mouse to pregnant mare serum gonadotrophins and human chorionic gonadotrophins. *J. Endocrinol.* **34:** 41-50.

McLaren, A. (1967) Factors affecting the variation in response of mice to goadotrophic hormones. *J. Endocrinol.* **37:** 147-154.

Wilson, E.D. and **Zarrow, M.X.** (1962) Comparison of superovulation in the immature mouse and rat. *J. Reprod. Fertil.* **3:** 148-158.

(Received 01/09/1992;
in revised form 24/04/1993)

الاختلافات بين سلالات من الفئران في كمية التبويض المنشّط بواسطة الهرمونات التناسليّة بيرجونال وبروفاسي

أحمد راشد الحميدي

قسم علم الحيوان - كلية العلوم - جامعة الملك سعود
ص.ب. ٢٤٥٥ - الرياض ١١٤٥١ - المملكة العربية السعودية

لقد تمّ في هذا البحث تحفيز ٩٠ أنثى من ثلاث سلالات من الفئران المخبريّة (Balb/c, CBA, C57BL/6) بواسطة الهرمونين التناسليّين بيرجونال (HMG) وبروفاسي (HCG) وبجرعات ثلاث من كلا الهرمونين (٥ و١٠ و١٥ وحدة دوليّة) وبمعدّل ١٠ إناث من كل سلالة لكل جرعة من الهرمونين. ولقد كانت تُحقَنُ جرعات HMG أولاً داخل التجويف البريتوني للإناث ثمّ تعقبها جرعات HCG بعد مرور ٤٨ ساعة، وبعد مرور ١٠ إلى ١٢ ساعة تُقتَل الإناث وتُزال منها قنوات البيض في أطباق بتري تحتوي محلول ملح طبيعي وذلك لازالة وعدّ البويضات. ولقد تمّ جمع ١٥٠٠ من البويضات، بمعدّل ١٦,٦ بويضة لكل أنثى. هذا ولقد أظهرت الدراسة اختلافات بين السلالات في مدى استجابتها للتحفيز بالهرمونات المستعملة حيث كانت استجابة إناث السلالة Balb/c هي الأفضل على الاطلاق عند الجرعة ١٠ وحدات دوليّة من الهرمونين واستجابة إناث السلالة CBA كانت جيّدة عند تلك الجرعات من الهرمونين، ولكن بصورة غير معنوية، هذا ولقد انخفضت استجابة إناث السلالتين سابقتَي الذكر عندما زيدت جرعات الهرمونين من ١٠ إلى ١٥ وحدة دولية. أمّا إناث السلالة C57BL/6 فلم تبدأ استجابتها للتحفيز بالهرمونين إلّا عند الجرعة ١٥ وحدة دوليّة لكلا الهرمونين.