Comparison of commercial gonadotrophins using bioassays

K.M. HENDERSON, A. WEAVER, R.L. WARDS, S. LUN AND K.P. McNATTY

Wallaceville Animal Research Centre, MAF Technology, P.O. Box 40063, Upper Hutt, New Zealand

ABSTRACT

The FSH:LH bioactivity ratios, determined by radioreceptor assays, of four commercial gonadotrophin preparations were Folligon 5; F.S.H.-P. 18; Folltropin 49 and Ovagen 1090. The mean number of oocytes produced in immature rats after ovulation induction following administration of these gonadotrophin preparations by injection (Folligon, 10 to 50 i.u.) or 48 h continuous infusion (others, 30 to 1000 μg/day) was dose-dependent, except at the highest doses when mean oocyte numbers either remained unchanged, or fell significantly in the cases of Folligon and F.S.H.-P. The highest mean number of oocytes produced in response to Folltropin (48±9) and Ovagen (47±7) were significantly higher ($P<0.05$) than those attained with Folligon (21±6) or F.S.H.-P. (31±5). Mean ovarian weights also increased in a dose-dependent fashion in response to each of the gonadotrophin preparations. Co-infusion of NIADDK-ovine LH-25 at 10 to 20 μg/day with Ovagen (250 μg/day) or NIADDK-ovine FSH-17 (10 μg/day) (both low in LH activity) increased mean oocyte production 1.5 to 3-fold. Co-infusion of ovine LH at 40 μg/day significantly reduced mean oocyte numbers.

Keywords Bioassay, rats, gonadotrophin, FSH, LH, oocytes, superovulation.

INTRODUCTION

Superovulation of farm animals is a widely used practice whereby exogenous gonadotrophins are used to increase the number of oocytes normally released at ovulation. These oocytes can be fertilized, and with the aid of surrogate mothers, large numbers of genetically superior offspring may be produced. A variety of superovulating gonadotrophin preparations are available for use. These are normally either serum gonadotrophins, e.g., pregnant mare's serum gonadotrophin (PMSG) or pituitary extracts of follicle stimulating hormone (FSH). Attempts to optimize the superovulatory response for different species has led to a variety of doses and administration regimes being recommended. However, making valid comparisons of different gonadotrophin preparations, doses and treatment regimes is difficult in farm animals because of the large numbers of animals and amounts of gonadotrophin required. Convenient bioassays which can provide objective information on the properties of gonadotrophin preparations are therefore sought. Radioreceptor assays have frequently been used to measure the FSH and luteinizing hormone (LH) bioactivity of gonadotrophins (Monniaux *et al.*, 1983; Murphy *et al.*, 1984; Donaldson *et al.*, 1986). However such *in vitro* bioassays may not always reflect the activity of a gonadotrophin *in vivo*.

In this study, an *in vivo* bioassay based on the ovulatory response of immature rats (Armstrong and Opavsky, 1988; Armstrong *et al.*, 1989) has been used to examine the superovulating attributes of 4 commercial gonadotrophin preparations (Folligon, F.S.H.-P., Folltropin and Ovagen). Folligon is prepared from the serum of pregnant mares, whereas the others are porcine (F.S.H.-P. and Folltropin) or ovine (Ovagen) pituitary extracts. In addition to FSH, these preparations also contain varying amounts of LH activity (Monniaux *et al.*, 1983; Murphy *et al.*, 1984; McNatty *et al.*, 1989).

MATERIALS AND METHODS

Gonadotrophin preparations

Folligon (batch no. 488121) and Chorulon (batch no. 690891) were obtained from Intervet (Aust) Pty Ltd, NSW, Australia. F.S.H.-P. (lot no. 548D86) was obtained from Burns-Biotec Laboratories, Nebraska, USA. Folltropin (lot V-028) was obtained from Vetrepharm Inc., London, Ontario, Canada. Ovagen (batch 8202) was obtained from Immuno-Chemical Products Ltd,

Auckland, New Zealand. Ovine FSH (NIADDK-oFSH-17; FSH activity 20 x NIH-FSH-S1 U/mg, LH activity 0.04 x NIH-LH-S1 U/mg) and ovine LH (NIADDK-oLH-25; LH activity 2.3 x NIH-LH-S1 U/mg, FSH contamination <0.5% by weight) were provided by the National Hormone and Pituitary Program, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, Maryland, USA. The protein content of the gonadotrophin preparations was determined by the method of Lowry *et al.*, (1951), using bovine serum albumin as the protein standard. The bioactive FSH and LH content of the gonadotrophin preparations was estimated from radioreceptor assays by the method of Cheng (1975). Membrane fractions, prepared by the method of Abou-Issa and Reichert (1977), from bovine testis and bovine corpora lutea were used as sources of FSH and LH receptor respectively.

Treatment of rats

Sprague-Dawley rats aged 21 to 24 days, obtained from the Experimental Small Animal Breeding Unit at Wallaceville, were used in the study. The rats were housed under constant temperature and light conditions, and were allowed pelleted food and water *ad libitum* throughout their treatment.

Folligon was administered in varying doses to rats 2 days before induction of ovulation (day 0) as a single subcutaneous injection in 0.2 ml saline. In preliminary experiments, varying doses of F.S.H.-P., Folltropin and Ovagen were also administered in 0.2 ml saline as a single subcutaneous injection or as once or twice (12 h apart) daily injections from day 0 to day 2. In later experiments, F.S.H.-P., Folltropin, Ovagen and the NIADDK preparations of ovine FSH and LH were administered by continuous infusion in saline using subcutaneously implanted Alzet mini-osmotic pumps (Model 2001, Alza Corporation, Palo Alto, California, USA). These pumps, which have a mean pumping rate of approximately 1 µl/h, were implanted into rats under light ether anaesthesia on day 0. On day 2, 48 h after either the first injection of gonadotrophin, or implantation of the pumps, the rats received a subcutaneous injection of 25 i.u. chorionic gonadotrophin (Chorulon) in 0.2 ml saline to induce ovulation. Twenty-four hours later, the rats were sacrificed by overdosing with pentobarbitone sodium. The ovaries and oviducts were excised and trimmed free of fat. The ovaries were blotted dry and weighed individually. Under a dissecting microscope, oocytes were released from the swollen ampullae of the oviducts into Dulbecco's phosphate buffered saline containing 250 µg/ml hyaluronidase (Type IV-S, Sigma Chemical Co., St Louis, MO, USA). Once the clumped cumulus-oocyte masses had been dispersed by the enzyme solution, the number of released oocytes was counted.

Data analysis

Ovarian weight data and oocyte number data were each summed for pairs of ovaries to give total values per rat. Mean and s.e.m. values were then calculated for each treatment group. One way analysis of variance in conjunction with the Newman-Keuls multiple range test was used to test the statistical significance of differences between mean values. The level of significance was set at $P<0.05$.

RESULTS

The units of all the gonadotrophin preparations differed, and so to provide a common parameter protein determinations were performed. The protein contents were: Folligon 0.3 µg/i.u;. F.S.H.-P. 0.6 mg/mg Armour standard; Folltropin 0.5 mg/mg NIH standard; Ovagen 32 mg/unit. The NIADDK ovine gonadotrophins contained >85% protein per unit weight.

Preliminary studies demonstrated that while immature rats could be induced to ovulate following a single injection of Folligon, ovulation could not be induced following administration of the other gonadotrophin preparations as either a single injection, or as once or twice daily injections, at total doses of up to 0.5 mg protein. Ovulation could, however, be induced following continuous infusion of F.S.H.-P., Folltropin or Ovagen via subcutaneously implanted mini-osmotic pumps. Figure 1 shows the effect of varying doses of the gonadotrophin preparations, given as either a single injection (Folligon) or continuous infusion (others), on ovarian weight and oocyte numbers.

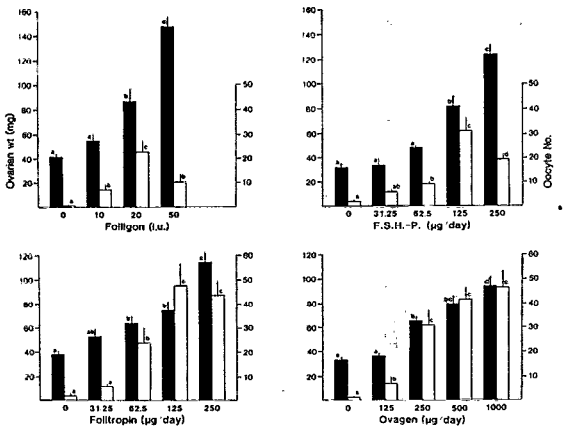

FIG 1 Effect of gonadotrophin preparations on ovarian weights and oocyte numbers. ■ mean ovarian weight; □ mean oocyte number.

Folligon doses are in international units, others are µg protein/day. Values are means of 5 rats/dose with vertical lines showing the s.e.m. Mean values with different letter superscripts indicate significant differences between mean ovarian weights or oocyte numbers ($P < 0.05$).

All the gonadotrophin preparations promoted a dose-dependent increase in mean ovarian weights. The mean number of oocytes produced also increased in a dose-dependent fashion, except at the highest doses. The highest doses of Folligon and F.S.H.-P. caused a significant reduction in mean oocyte numbers, relative to the second highest doses, although mean ovarian weights continued to rise significantly. The top doses of Folltropin and Ovagen had no significant effect on mean oocyte numbers, relative to the second highest doses. Table 1 shows the highest mean numbers of oocytes produced in response to each of the 4 gonadotrophin preparations, together with the ratios of the FSH to LH bioactivity of the gonadotrophins, as determined by radioreceptor assays. The highest mean number of oocytes were produced in response to Folltropin and Ovagen. These two gonadotrophins also had higher FSH:LH ratios than either F.S.H.-P. or Folligon. Interestingly, although there was a 20-fold difference in the FSH:LH ratio of Folltropin and Ovagen, the highest mean numbers of oocytes produced in response to each were similar.

TABLE 1 Oocyte production and FSH:LH bioactivity ratio of the gonadotrophin preparations

Gonadotrophin preparation	Dose producing highest mean oocyte no.	Highest mean oocyte no. ± s.e.m.	FSH:LH bioactivity ratio
Folligon	20 i.u.	21±6[a]	5
F.S.H.-P.	125 µg/day	31±5[a]	18
Folltropin	125 µg/day	48±9[b]	49
Ovagen	1000 µg/day	47±7[b]	1090

Mean values with different letter superscripts are significantly different, $P<0.05$, $N=5$
Folligon dosage is in international units, others are µg protein/day

To investigate further the possibility that the ovarian response to the gonadotrophin preparations was influenced by their FSH:LH ratios, the effect of co-infusing NIADDK- ovine LH-25 with either Ovagen or NIADDK-ovine FSH-17 (both preparations low in LH activity) was studied. The results are shown in Figure 2. Relative to infusion with 250 µg Ovagen/day alone, co-infusion with 10 µg LH/day increased mean ovarian weight significantly (from 52 ± 2 to 98 ± 4 mg), and increased mean oocyte numbers (from 21 ± 6 to 38 ± 10), though this increase was not statistically significant. Increasing the dose of LH to 20 µg/day caused a further significant increase in the mean ovarian weight (to 122 ± 13 mg) and a significant increase in mean oocyte numbers (to 58 ± 9). With the latter regime, the mean oocyte number and mean ovarian weight was comparable to that achieved with the highest dose of Folltropin (44 ± 6 oocytes, 115 ± 8 mg ovary; Fig. 1). Further increasing the dose of LH to 40 µg/day significantly reduced the mean oocyte number to 23 ± 2, but had no significant effect on mean ovarian weight (116 ± 6 mg). These values were similar to those produced by the highest dose of F.S.H.-P. (19 ± 2 oocytes, 124 ± 8 mg ovary; Figure 1).

Infusion of 20 µg LH/day with 10 µg NIADDK ovine FSH/day significantly increased both mean oocyte number and ovarian weight approximately 1.5-fold, relative to infusion of FSH alone. Increasing the dose of LH to 40 µg/day had no further effect on mean ovarian weight, but significantly reduced the mean oocyte number to that achieved with FSH alone. Infu-

sion of LH on its own at doses from 10 to 40 μg/day had no effect on mean oocyte numbers or ovarian weight relative to non-infused control rats.

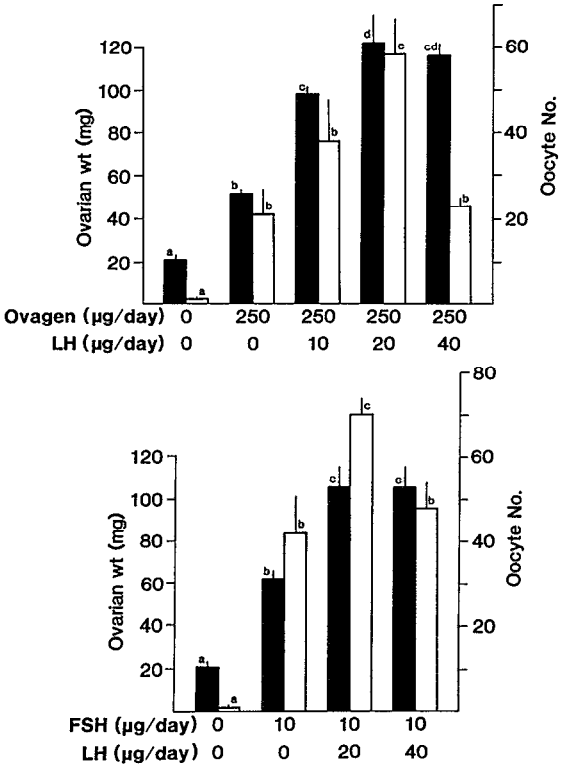

FIG 2 Effect of co-infusion of NIADDK-ovine LH-25 with Ovagen or NIADDK-ovine FSH-17 on ovarian weights and oocyte numbers. ■ mean ovarian weight; ☐ mean oocyte number.

Doses are μg protein/day. Values are means of 5 rats/dose with vertical lines showing the s.e.m. Mean values with different letter superscripts indicate significant differences between mean ovarian weights or oocyte numbers ($P < 0.05$).

DISCUSSION

This study shows that the ovulatory response of immature rats is a useful additional bioassay to study the attributes of novel and established gonadotrophin preparations. However, it must be emphasised that while bioassays such as this and others, e.g., receptor assays, can provide useful information on the relative properties of gonadotrophin preparations, they cannot be used to accurately predict the ovulatory response in any particular species. Proper follow-up trials to assess superovulatory effectiveness in the species of choice remain essential.

There were marked differences in the ovarian responses to the four commercial gonadotrophin preparations studied. This may be related, at least in part, to differences in the ratio of the bioactive concentrations of FSH and LH between the preparations. Ovagen and Folltropin which had the highest FSH:LH ratios also produced the highest mean number of oocytes. The importance of the FSH:LH ratio was also demonstrated in the results of co-infusing LH with Ovagen or NIADDK-ovine FSH, which are both low in LH content. Co-infusion of low doses of LH (10-20 μg/day) increased the mean numbers of oocytes produced in response to Ovagen and NIADDK-ovine FSH. High doses of LH (40 μg/day), in contrast caused a reduction in mean oocyte numbers, relative to the effects of low doses of LH. Thus while the addition of LH to gonadotrophin preparations low in LH activity may enhance the ovulatory response, raising the LH component too much can have a deleterious effect. Similar findings have also recently been reported by Armstrong *et al.*, (1989). The optimum FSH:LH ratio for superovulation is, however, likely to vary from species to species of recipient animal, or even between breeds (Chupin *et al.*, 1985), and may also be dependent on the species from which the gonadotrophin is prepared.

Although all the gonadotrophin preparations produced a dose-dependent increase in mean ovarian weights, the weight increase was not always accompanied by increased mean oocyte production. At the highest doses of the gonadotrophin preparations, increases in mean ovarian weights were accompanied by either no change or a fall in the mean numbers of oocytes produced. Thus, bioassays based on increases in ovarian weight alone may not give a good indication of the superovulatory potency of gonadotrophin preparations. Armstrong *et al.*, (1989) have also reached a similar conclusion.

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