MEDWELL PUBLISHING
Journal of Animal and Veterinary Advances 8 (6): 1219-1221, 2009
ISSN: 1680-5593

Effect of Different Protocol of Ovarian Stimulation, with Gonadotrophin on Concentration of Ovarian Hormones

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Abstract: The present study was undertaken to determine whether different protocol of gonadotropin, involved in regulation of ovarian steroidogenesis in female mice. The purpose of the present study was to determine the better time of treatment with gonadotropin. Thirty female mice in age from 4-5 weeks was used. All animal received 10 Iu gonadotropin (IP), then animals divided in 5 groups (n = 6 per group) group 1 = Control (primer injection), group 2 after 2 weeks, group 3 after 4 weeks group 4 after 6 weeks and group 5 after 8 weeks after primer injection super ovulated or stimulated by injection gonadotropin (10 IU, IP). In each group, 46 h after injection, concentration of estrogen and progesterone were measured by Electrochemiluminescenc (ECL). Blood estrogen level decrease in group 2 and blood estrogen and progesterone. Concentration in group 3 was higher than other groups, the results of this study confirmed that the best time for treatment with gonadotrophin according ovarian hormones for IVF is 4 weeks after primer injection (group 1).

Key words: Gonadotropin, estrogen, progesterone, ovary, ECL, IP

INTRODUCTION

Extending the FSH window for multi follicular development by administering FSH from the midfollicular phase onward constitutes a novel mild protocol for ovarian stimulation for *In vitro* Fertilization (IVF) based on the physiology of single dominant follicle selection in normo-ovulatory women (Femke *et al*., 2002). Repeated treatment with the same gonadotrophin has been found to cause a decrease in the super ovulatory response in rabbits (Maurer *et al*., 1968), Sheep (Palsson, 1962), Calves (Howe *et al*., 1962) and cows (Turman *et al*., 1977; Turman and Wetteman, 1978). This decreased response has been attributed to refractoriness resulting in part from formation of antibodies against exogenous gonadotrophins (Willett *et al*., 1953; Jainudeen *et al*., 1966; Onuma *et al*., 1969). In contrast, other studies have not shown a reduction in the response of cows to repeated treatment with the same gonadotrophin (Dziuk *et al*., 1958) or ewes (Gordon, 1975).

From the result's of studies (Lubbadeh *et al*., 1980), it is unclear whether repeated treatment with the same gonadotrophin Lead to a decrease in the ovarian activity and secretion the ovarian hormones, because of immunological reasons. The present experiment was undertaken to determine the superovulatory response and secretion ovarian hormones.

MATERIALS AND METHODS

Thirty female mice aged 4-5 weeks were used in present study animals were housed in plastic cages under standard condition with free access to drinking water and basal diet. The animals were adapted to the laboratory condition for 7 days before the experimental procedure, They were maintained in a room with controller temperature (20-22°C), relative humidity (50%) and 14 h light/10 h dark cycle after 7 days adaptation. All animals were super ovulated with (gonadotropin) (10 IU, IP) then they were divided in 5 groups:

Group 1: Control (primer injection, single injection).

Group 2: Two weeks after primer injection, treatment with gonadotropin in proestrus phase (10 Iu).

Group 3: Four weeks after primer injection, treatment with gonadotrophin in proestrus phase (10 Iu).

Group 4: Six weeks after primer injection, treatment with gonadotrophin in proestrus phase (10 Iu).

Group 5: Eight weeks after primer injection, treatment with gonadotrophin in proestrus phase (10 Iu).

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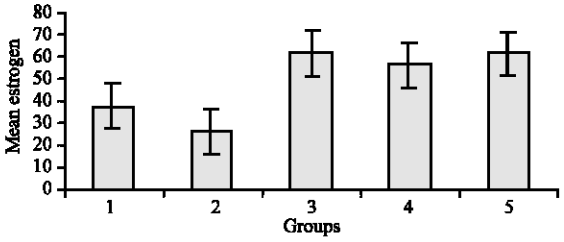
At the end of each period the animals were anaesthetized by diethylether and venous blood samples were collected by direct heart puncture, blood was centrifuged then the concentration of estrogen and progesterone was performed by Electrochemi-luminescense (ECL).

Statistical analysis: The statistical analysis was performed by SPSS continuous data expressed as mean±SD. Data were compared a sing one-way ANOVA, $p<0.05$ was considered to be statistically significant.

RESULTS

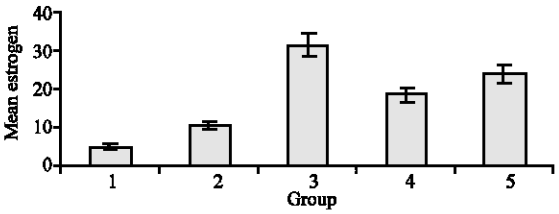
The results show that blood estrogen level decrease in group 2 and administration of gonadotrophin 4 weeks after primer injection (group 1) increased plasma estrogen (Fig. 1) (group 3) as result indicated there was a significant increase ($p<0.05$) in blood estrogen level in mice treated with gonadotrophin after 4 weeks compared to control, the results show that the blood progesterone level increase in group 3 (Fig. 2). The results of the present study confirmed that in mice treatment with gonadotrophin 4 weeks after primer injection the blood estrogen and progesterone concentration was higher than other groups (Fig. 3). There was a slight decrease in estrogen and progesterone level in groups 4 and 5 as compared to the group 3 although not significant, When considering the progesterone level. Decrease is more obvious (Fig. 3).



			Sub set for α = 0.5	
	Groups	N	1	2
Duncan[a]	2	6	25.7567	
	1	6	36.7633	55.3133
	4	6		60.2333
	5	6		60.3833
	3	6		0.475
	Sig.		0.122	

Means for groups in homogeneous subsets are displayed; [a]Uses hormonic mean sample size = 3.000

Fig. 1: Effect of gonadotrophin on ovarian estrogen synthesis



			Sub set for α = 0.5		
	Groups	N	1	2	3
Duncan[a]	2	6	4.4567		
	1	6	10.1633	10.1633	
	4	6	18.2733	18.2733	18.2733
	5	6	36.7633	23.9367	23.9367
	3	6			31.1933
	Sig.		0.098	0.099	0.119

Means for groups in homogeneous subsets are displayed; [a]Uses hormonic mean sample size = 3.000

Fig. 2: Effect of gonadotrophin on ovarian progesterone synthesis

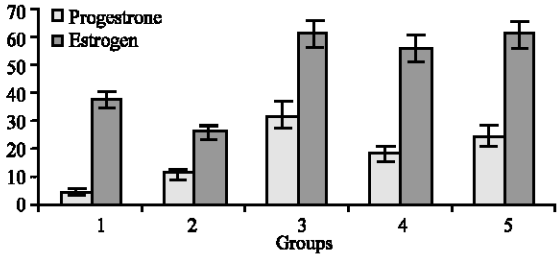


Fig. 3: Effect of gonadotropin on ovarian estrogen and progesterone synthesis

DISCUSSION

It has been 25 years since the introduction of *in vitro* Fertilization (IVF) for treatment of infertility (Baczkowski *et al*., 2004) during this time very dynamic advances have taken place in all aspects of Assisted Reproductive Technology (ART) (Maurer *et al*., 1968). In all the methods, selection the best protocol of treatment with gonadotropine is very important (Baczkowski *et al*., 2004). Reviews concerning repeated superovulation of cows with the same gonadotrophin indicated that the ovarian response was decreased in the subsequent treatment (Foote and Onuma, 19369; Gordon, 1975; Willet *et al*., 1953; Onuma *et al*., 1969). Repeated that this decrease in the response of mice in the 2nd superovulation period continued, however, in the present study there was no decrease in repeated superovulation in the group 3 in mice. But on the contrary there was a slight decrease in groups 4 and 5 compared to the group 3. Although there was a significant decrease in estrogen and progesterone level in group 2, the response observed in the group 3 indicated that no immunological

refractoriness developed. The most probable reason for the decrease in the group 2 was the short time interval between the successive superovulations in these wo groups. The short interval between groups 2 and 1 gonadotrophin injection might also have been partially the reason behind the decrease response observed in the group 2. The increase response in group 3 supports these suggestions.

CONCLUSION

These results indicated that repeated super-ovulations with the same gonadotropin for 5 successive times within 60 days did not lead to a decreased response throughout the 5 superovulated period. Further studies with multiple super ovulations encompassing longer periods are needed.

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