An in vitro Comparative Study of Follicle Stimulating Hormone (FSH) and Activin A Effects on the Maturation of Preantral Follicle-Enclosed Oocytes from Immature Syrian Mice

Aisha Javed1 & 2, Amer Jamil2, Saeed Rezaei-Zarchi3, Seyed Mehdi Kalantar*1 & 4, Morteza Anvari1 and Habib Nazem5

1Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Sciences, Yazd, Iran; 2Molecular Biochemistry Lab, Dept. of Biochemistry, University of Agriculture, Faisalabad, Pakistan; 3Dept. of Biology, Payam-e-Noor University of Taft, Yazd, Iran; 4Yazd Medical Biotechnology and Genetic Engineering Incubator, Yazd, Iran; 5Central Payam-e-Noor University, Tehran, Iran

Received 29 May 2007; revised 23 September 2007; accepted 29 September 2007

ABSTRACT

Objectives: It was aimed to investigate the effects of different doses of follicle stimulating hormone (FSH) and activin A on the growth and maturation of preantral mouse follicles during the in vitro culture. Methods: Preantral follicles (90-100 µm in diameter) were harvested from 6-8 week-old Syrian mice and cultured in TCM199 culture medium for 6 days to see the effect of FSH and Activin A. Activin A concentrations in the range of 10-200 ng/ml were used, while 10, 50, 100, 150 and 200 mIU/ml FSH were used in the experiment. Results: Activin A concentration of 100 ng/ml resulted in a significant increase in follicle diameter (170 µm) with the survival rate of 73% as compared to the control (100 µm and 25%, P<0.05). The number of oocytes matured and the percentage of germinal vesicle breakdown (GVBD) was 61 and 70%, respectively as compared to the control (20 and 29%, P<0.05). Follicle diameter (190 µm) and survival rate (85%) increased significantly in the presence of 100 mIU/ml of FSH as compared to the control (P<0.05). But, the administration of activin A+FSH increased the effect of both factors on follicular diameter (205 µm as compared to 100 µm in control, P<0.01). Follicle survival, oocyte maturation and GVBD rates were 91, 81 and 89%, respectively (P<0.01). Conclusion: These results have suggested that exposure to FSH and activin A before the formation of antral cavity had positive effect on follicle survival and oocyte robustness. Iran. Biomed. J. 12 (2): 85-92, 2008

Keywords: Follicle stimulating hormone (FSH), Oocyte maturation, Follicle, Activin A, Germinal vesicle breakdown (GVBD)

INTRODUCTION

Oocyte maturation is defined as the reinitiating and completion of the first meiotic division, subsequent progression to metaphase II, and the nuclear and cytoplasmic processes, which become essential for fertilization and early embryo development. In vitro animal models provided insight into the importance of substances affecting oocyte maturation and its inhibition, such as cAMP, calcium, cell-cycle proteins, growth factors, GnRH, gonadotropins, purines, and steroids. Vertebrate oocytes are arrested at prophase I of meiosis, during which they undergo a lengthy period of growth. Meiosis is resumed during the final oocyte maturation, which is initiated by a surge release of gonadotropins, especially follicle stimulating hormone (FSH) and LH, from the pituitary [1].

FSH is essential for the steroidogenesis by stimulating aromatase enzyme activity (P450 aromatase), for differentiation of the granulosa cells (GC). FSH also regulates the transzonal connection between the oocytes and the surrounding GC [2]. Furthermore, the presence of gonadotropins induces the expression of inhibitor of apoptosis proteins (IAP) by GC in vivo and in vitro [3]. Finally, FSH interacts with several growth factors to induce follicular growth such as kit ligand, EGF, activin A, inhibin, BMP-15 or insulin-like growth factor. These
intra-ovarian regulators mediate the effect of gonadotropins in regulating cellular interactions by autocrine and paracrine mechanisms [4]. FSH is assumed to promote signals for GVBD induction to a much greater extent in cumulus-enclosed oocytes than in cumulus-denuded oocytes. This would mean that maintenance of gap-junction communication between cumulus cells and the oocyte is essential for FSH stimulation of maturation [5]. Endocrine control of follicular development by FSH rests on a network of intrafollicular paracrine interactions [6]. For example, FSH promotes proliferation and differentiation of preantral follicles via paracrine factors such as activin [7].

Although pituitary gonadotropins are critical in inducing oocyte maturation in all vertebrates, the increasing evidence also suggests important roles for the local ovarian factors such as steroids and non-steroidal substances in the event. A major group of non-steroidal substances that are implicated in the regulation of ovarian functions, including final oocyte maturation, is the activin/inhibin family of growth factors. Activin A is a dimeric protein consisting of two similar but distinct subunits, βA and βB. Activin A is structurally related proteins that belong to the multi-functional transforming growth factor b family. Although initially recognized as an ovarian protein that stimulates the secretion of pituitary FSH [2], activin has been shown to have diverse biological activities in a variety of tissues [3].

Activin is expressed in ovarian cells during follicular development and it plays a stimulatory role during early follicular development and in oocytes and GC development from the preantral stage. It plays an autocrine/paracrine role in controlling early follicular development by promoting follicular growth and differentiation [8, 9]. Activin treatment results in the formation of follicular structures in cultures of dispersed rat ovary containing oocytes. Activin from GC signals to oocytes to produce one or more activities that are necessary for follicle development [10, 11].

In vitro, activin enhances aromatase activity and suppresses FSH-induced progesterone synthesis in rat GC. Therefore, it has been proposed as a local modulator of GC steroidogenesis [12]. Li et al. [13] reported that activin promoted the in vitro development of theca-free granulosa-oocyte complexes isolated from the follicles. Similarly, Yokota et al. [14] reported that activin A had a stimulatory effect on cultured, intact preantral follicles recovered from immature mice. The potential role of activin in the regulation of oocyte maturation has been investigated in a number of mammalian species. It has been demonstrated that activin A promotes in vitro oocyte maturation in the rat, cow, rhesus monkey [15], and human [16].

The present study was aimed to clarify the physiological significance of FSH and activin on the in vitro follicular growth of preantral follicles obtained from immature Syrian mice. Furthermore, combined effect of the two factors was also elucidated in the recent study. To assess the quality of cultured follicles, emphasis was put on detailed structural differences in follicles cultured in the presence or absence of activin A. In addition, the effect of FSH and combined effect activin A and FSH was also studied on the following: 1) follicle diameter and survival rate, and 2) the percentage of oocytes matured and germinal vesicle breakdown (GVBD).

**MATERIALS AND METHODS**

**Chemicals.** Recombinant human FSH (rhFSH) was obtained from Organon Co. (Oss, North Brabant Province, the Netherlands). Human erythroid differentiation factor/activin A (activin A, βAβA) was provided by Genentech Inc. (South San Francisco, CA, USA). All other chemicals were of analytical grade or the highest quality commercially available.

**Animal model for follicle recruitment.** Female Syrian mice were housed and bred in Central Animal House of Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Sciences, Yazd, Iran. Animals were kept under controlled conditions with 14 hours light/12 hours dark photoperiod, and fed with water and food pellets ad libitum. Six to eight weeks mice were used for the isolation of cumulus enclosed oocytes as described by Mahmoudi et al. [17]. The animals were killed by cervical dislocation after 44-48 hours of stimulation by an i.p. injection of 7.5 IU per mouse pregnant mare's serum gonadotrophin.

**Follicle culture.** For preantral follicles, the ovaries were removed aseptically and placed in Falcon plastic Petri dishes (Falcon 3037, Becton Dickinson and Co., Rutherford, NJ, USA) filled at room temperature with α-MEM (GIBCO BRL, Tokyo, Japan). After removing the surrounding tissue, the ovaries were micro-dissected using two 27-gauge needles attached to 1-ml syringes under

---

**MATERIALS AND METHODS**

**Chemicals.** Recombinant human FSH (rhFSH) was obtained from Organon Co. (Oss, North Brabant Province, the Netherlands). Human erythroid differentiation factor/activin A (activin A, βAβA) was provided by Genentech Inc. (South San Francisco, CA, USA). All other chemicals were of analytical grade or the highest quality commercially available.

**Animal model for follicle recruitment.** Female Syrian mice were housed and bred in Central Animal House of Research and Clinical Center for Infertility, Shahid Sadoughi University of Medical Sciences, Yazd, Iran. Animals were kept under controlled conditions with 14 hours light/12 hours dark photoperiod, and fed with water and food pellets ad libitum. Six to eight weeks mice were used for the isolation of cumulus enclosed oocytes as described by Mahmoudi et al. [17]. The animals were killed by cervical dislocation after 44-48 hours of stimulation by an i.p. injection of 7.5 IU per mouse pregnant mare's serum gonadotrophin.

**Follicle culture.** For preantral follicles, the ovaries were removed aseptically and placed in Falcon plastic Petri dishes (Falcon 3037, Becton Dickinson and Co., Rutherford, NJ, USA) filled at room temperature with α-MEM (GIBCO BRL, Tokyo, Japan). After removing the surrounding tissue, the ovaries were micro-dissected using two 27-gauge needles attached to 1-ml syringes under
the stereomicroscope and preantral follicles (100 µm in diameter) with one or two layers of GC around the oocyte and an intact basal lamina with thecal cells were mechanically isolated [18]. To test the effects of FSH and activin A, 30 preantral follicles were transferred into a Falcon plastic Petri dish filled with 1 ml serum-free DMEM supplemented with 6.25 mg/ml insulin, 6.25 mg/ml transferrin, 6.25 ng/ml selenium acid, 5.35 mg/ml linoleic acid, 0.15% BSA, 15 mM HEPES, 45 mg/ml penicillin G, 350 g/ml streptomycin, and 1.75 mg/ml amphotericin, 10, 25, 50, 75, 100 and 200 ng/ml activin A, while 10, 50, 100, 150 and 200 mIU/ml FSH. The follicles were cultured in a humidified chamber with 5% CO₂ in the air at 37°C for 6 days. Each experiment was repeated 5–6 times and media were prepared freshly at two days interval. Activin A and rhFSH were added on day 0 at the indicated concentrations. Follicles cultured with the medium alone served as the control.

**In vitro maturation of preantral follicle-enclosed oocytes.** Within 60 minutes of collection, follicle-enclosed oocytes were assigned randomly to the culture conditions and incubated individually in 50-µl drops under 5 ml of TALP-equilibrated, sterile mineral oil in a humidified atmosphere of 5% CO₂ in air at 37°C [16]. The oocytes complexes were evaluated in a blinded fashion with regard to the treatment conditions using an inverted microscope with Hoffman optics (×40 at the end of day-6 culture period) for cumulus expansion and mucification [19]. The medium was refreshed by changing half of the quantity every other day.

**Measurements and statistics.** Histological measurements and observations were made under the inverted microscope with a crossed micrometer (IMT-2, Olympus Corp., Tokyo, Japan). At the end of incubation, oocytes were observed by inverted microscopy and morphological changes in the nucleus or the extrusion of first polar body, during (meiosis phase II were used as the criterion for nuclear maturation of GV-stage oocytes. Only the follicles, which had maintained basement membrane integrity during the culture, were said to be survived and used for further analysis [8, 17]. Maximum and minimum lengths of each follicle were measured daily with an inverted microscope (IMT-2, Olympus Corp., Tokyo, Japan). Interstitial and thecal cells around the basement membrane were not included in the measurement of the follicle [15]. The mean diameter of the follicle was calculated by averaging these two measurements. The influence of the peptides on the extent of oocyte maturation and an increase in follicle diameter, and comparison of each group with the control was determined by comparing percentages using one-way analysis of variance for repeated measures to determine significant differences among the group means [18]. *P*<0.05 was considered significant.

**RESULTS**

**Effect of different concentrations of activin A on the survival rate and diameter of the follicles.** To ensure the effect of activin concentration, the experiments were conducted assessing the follicle diameter and survival rate in the presence of a particular concentration of experimental protein. Changes in survival rate and diameter of preantral follicles, obtained from 56-day-old Syrian mice, are presented in Figure 1. Follicle diameter increased by increasing the concentration of activin A. Figure 1 shows the effect of 10, 25, 50, 75 and 100 ng/ml of activin A on the follicle survival percentage and diameter. Follicles cultured in the presence of 10, 25, 50 and 75 µg/ml of activin A did not show any significance difference in diameter (*P*<0.05).
Fig. 2. Effect of different concentrations of FSH on follicle diameter (µm) and survival rate. Preantral follicles with a mean diameter of 100-120 µm were cultured for 6 days in TCM199 medium alone (control) and in the presence of 10, 50, 100, 150 and 200 mIU/ml of FSH. Follicle diameters (shown by ▲ in the Figure) and survival rates (shown by ● in the Figure) were checked every day and degenerated follicles were removed from the medium. n = 30 (total number of follicles in each experiment).

However, the follicles grown in the presence of 100 ng/ml of the protein showed a significance increase in follicle diameter up to 170 µm as compared to the control (100 µm) where, \(P<0.05\). Activin-effect on follicle survival rate revealed that all the concentrations of activin, used in the experiment, did not have any regulatory or inhibitory effect on follicle survival rate as compared to the control.

**Effect of different concentrations of recombinant FSH on the survival rate and diameter of follicles.**

To ensure the effect of FSH concentration, the experiments were conducted assessing the follicle diameter in the presence of a particular concentration of experimental protein. Follicle diameter increased by increasing the concentration of FSH. Figure 2 shows the effect of 10, 50, 100, 150 and 200 mIU/ml of FSH on the follicle diameter and survival rate. Follicles cultured in the presence of 10, 50, 150 and 200 mIU/ml of FSH did not show any significance increase in diameter. However, the follicles grown in the presence of 100 mIU/ml of the protein, showed a significant increase in follicle diameter up to 190 µm as compared to the control (\(P<0.05\)).

**Combined and comparative effects of activin A and recombinant FSH on the survival rate and diameter of follicles.** Figure 3 shows changes in the diameter and survival rate of the follicles cultured with activin A (100 ng/ml), rhFSH (100 mIU/ml), and a combination of both. Control follicles were cultured with the medium alone. As shown in Figure 3, preantral follicles cultured in medium alone showed no significant increase in diameter during the 6-day period while, a progressive increase in size was seen in response to activin A+FSH treatment, and the difference from the control was significant (\(P<0.01\)). The results obtained in the experiment show that FSH had a synergistic effect with activin A. The size of preantral follicles cultured in the presence of both activin A and FSH was nearly twice that of the control.

**Effect of different concentrations of activin A on the in vitro oocyte-maturation and GVBD percentage.** To conduct definitive experiments of oocyte maturation and GVBD changes with activin, 10, 25, 50, 75, 100 and 200 ng/ml of activin A was added to the culture medium of 30 follicle-enclosed oocytes recovered from ovaries of four females (i.e., four experiments conducted for 6 days of culture). In *vitro* maturation of oocytes, in the presence of 100 ng/ml of activin showed a significant number of matured oocytes as compared to control and other concentrations of activin used. Figure 4 shows that the oocytes cultured in 10, 50, 75 and 200 ng/ml of activin did not alter GVBD and oocyte maturation significantly compared with the control. However,
100 ng/ml of the protein added to the culture medium caused an increase in the number of mature oocytes as well as GVBD was noted to be 70% in this experiment as compared to the control ($P<0.05$). Culturing oocytes beyond 100 ng/ml did not result any significant changes in oocyte maturation. Therefore, all subsequent experiments were conducted with a medium supplemented with 100 ng/ml activin A.

**Fig. 4.** Effect of activin on oocyte maturation and germinal vesicle breakdown (GVBD) rate in 6 days culture. Preantral follicles with a mean diameter of 100-120 µm were cultured for 6 days in TCM199 medium alone (control) and in the presence of 10, 25, 50, 75, 100 and 200 ng/ml of activin A. The rate of oocyte maturation (shown by ● in the Fig.) and GVBD (shown by ■ in the Fig.) was checked every day and degenerated cells were removed from the medium. $n = 30$ (total number of follicles in each experiment).

**Effect of different concentrations of recombinant FSH on the in vitro oocyte-maturation and GVBD percentage.** Similar experiment was conducted with 10, 50, 100, 150 and 200 mIU/ml of recombinant FSH, added to the culture medium of 30 follicle-enclosed oocytes recovered from ovaries of four females (*i.e.*, four experiments conducted for 6 days of culture). Figure 5 shows that the oocytes cultured in 10, 50, 150 and 200 mIU/ml of FSH did not alter GVBD and oocyte maturation significantly compared with the control. However, 100 mIU/ml of FSH added to the culture medium caused an increase in the number of mature oocytes as well as GVBD was noted to be 74% in this experiment as compared to the control ($P<0.05$). Culturing oocytes beyond 100 mIU/ml did not result any significant changes in oocyte maturation, therefore, all subsequent experiments were conducted with a medium supplemented with 100 mIU/ml of FSH.

**Fig. 5.** Effect of different concentrations of FSH on oocyte maturation and germinal vesicle breakdown (GVBD). Preantral follicles with a mean diameter of 100-120 µm were cultured for 6 days in TCM199 medium alone (control) and in the presence of 10, 50, 100, 150 and 200 mIU/ml of FSH. Rate of oocyte maturation (represented by ● in the Figure) and GVBD (shown by ■ in the Figure) was checked every day and degenerated cells were removed from the medium. $n = 30$ (total number of follicles in each experiment).

**Combined and comparative effects of activin A and recombinant FSH on the in vitro oocyte-maturation and GVBD percentage.** To see the combined effect of FSH and activin on *in vitro* maturation of oocytes and GVBD, the study was conducted with optimum FSH (100 mIU/ml) and activin A (100 ng/ml) for 6 days culture of 30 preantral follicle-enclosed oocytes. Figure 6 shows...
the combined effect of FSH and activin on the percentage of oocyte maturation and GVBD as compared to the control experiment. In the presence of FSH + activin A, 81% oocytes matured with an 89% GVBD as compared to 20 and 45% oocyte maturation rate and GVBD percentage, respectively (P<0.01). As shown in Table 1, a combined treatment of FSH and activin A significantly increased the follicles diameter, survival and oocyte maturation rate, and GV break down.

**DISCUSSION**

The follicle population used in this study is the preantral follicle consisting of an oocyte surrounded by several layers of follicle cells and the theca layer. These components are necessary for follicular development and are interactive [20], and folliculogenesis cannot be reproduced in a physiological manner in the absence of one or more component. Such an *in vitro* enclosed follicle culture system is a better method for elucidating and understanding folliculogenesis. Folliculogenesis is a series of physiological events defined by morphological and functional changes of the follicle. Of these events, antrum formation is considered the mile-stone of folliculogenesis, and a number of attempts have been made to produce antrum formation from preantral follicles, with limited success [21].

The growth of follicles after antrum formation is undoubtedly regulated by the pituitary gonadotropin FSH [22]. FSH is involved in early folliculogenesis, up to the antrum formation stage. FSH stimulates follicular maturation and differentiation via membrane-bound receptors coupled to cAMP post-receptor signaling [8]. FSH is essential for the steroidogenesis by stimulating aromatase enzyme activity (P450 aromatase), for the differentiation of the GC by inducing the expression of LH receptors and for the follicular antrum formation. FSH also regulates the transzonal connection between the oocytes and the surrounding GC [2]. Furthermore, the presence of gonadotropins induces the expression of IAP by GC *in vivo* and *in vitro* [3].

The fact that FSH treatment increases the number of preantral and small antral follicles in mouse further supports that follicular growth up to antrum formation is controlled by factors other than FSH such as, activin proteins [23]. Nayudu and Osborn [21] have succeeded in inducing antrum formation with FSH in a dose-related manner in an *in vitro* follicle culture system using preantral follicles from 42- and 56-day-old mice [21]. In our experiment, activin A produced a significant increase in follicular size, indicating that activin A is a local regulator that stimulates folliculogenesis in the preantral follicles. Activin A executes its actions through a group of intracellular signal transducers [8, 24]. Activin A is capable of directly influencing early oocyte and follicle development. The presence of receptors on both the oocyte and somatic cells may provide an explanation for the dose-dependent effect of activin on oocyte growth presented here. Moreover, activin did not promote inappropriate differentiation during the culture period. However, FSH alone had a significant effect on the follicle diameter and survival rate (190 and 71%, respectively) of preantral follicles (Fig. 2). FSH also showed a significant synergistic effect with activin A (Fig. 3). These results were consistent with those of Li *et al.* [13], who demonstrated that activin A, and FSH, stimulates morphological changes in the GC-oocyte complexes obtained from 56-day-old mice [13]. These results indicate that activin A together with FSH promoted the functional development of cultured follicles as well. It has been shown that activin A induces FSH receptors *in vitro* GC culture system of mice. Therefore, it is suggested that the synergistic effect of activin A and FSH may be mediated by the effect of activin A to increase a number of FSH receptors in the preantral follicle as well as to stimulate the differentiation of the follicle cells [25, 26].

**Table 1.** Comparative effects of FSH and activin A on follicle diameter, survival percentage, oocyte maturation rate and GVBD (%) during 6 days of culture.

<table>
<thead>
<tr>
<th>Treatment group/ml</th>
<th>Age (days)</th>
<th>No. of exp.</th>
<th>Follicle diameter (µm)</th>
<th>Follicle survival rate (%)</th>
<th>Oocyte maturation rate (%)</th>
<th>GVBD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>56</td>
<td>5</td>
<td>100</td>
<td>25</td>
<td>20</td>
<td>29</td>
</tr>
<tr>
<td>100 ng Activin A</td>
<td>56</td>
<td>5</td>
<td>170</td>
<td>73&lt;sup&gt;a&lt;/sup&gt;</td>
<td>61&lt;sup&gt;b&lt;/sup&gt;</td>
<td>70&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>100 mIU FSH</td>
<td>56</td>
<td>5</td>
<td>190&lt;sup&gt;b&lt;/sup&gt;</td>
<td>85&lt;sup&gt;b&lt;/sup&gt;</td>
<td>73&lt;sup&gt;b&lt;/sup&gt;</td>
<td>85&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>100 mIU FSH + 100 ng Activin A</td>
<td>56</td>
<td>5</td>
<td>205&lt;sup&gt;c&lt;/sup&gt;</td>
<td>91&lt;sup&gt;c&lt;/sup&gt;</td>
<td>81&lt;sup&gt;c&lt;/sup&gt;</td>
<td>89&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are the mean ± SEM; *P*<0.05 vs. control group; *P*<0.05 vs. control group; *P*<0.01 vs. control group.
On the other hand, FSH can induce its own receptors in GC [4, 9-11], but whether this action of FSH is a direct effect or because of other factors such as activin A, still remains unclarified. FSH induces the follicular diameter at specific concentrations but beyond these concentrations, it does not have a significant positive effect. Figure 2 shows that at 100 mIU/ml concentration, FSH has a significant effect on follicle diameter and survival rate but the concentrations beyond this have a negative effect as compared to 100 mIU/ml. According to some previous works, every follicle has FSH receptors along with the receptors of other gonadotropins. FSH stimulates the follicular maturation and differentiation via membrane-bound receptors coupled to cAMP post-receptor signaling and the excessive exposure to FSH could result in receptor down-regulation, leading to a suboptimal follicular response.

Compatible with previous reports using follicles from adult hamsters, FSH stimulated follicular growth in adult mice. However, there was a marked contrast between the growth of follicles from adult mice and immature mice. Of particular interest is the effect of FSH, which was enhanced by co-treatment with activin A [24, 27, 28].

More follicles start to grow per day in the immature mouse and rat than in older animals, probably due to high levels of FSH in the prepubertal rodent. Most follicles in the ovary of the immature mouse (11-days-old) are at the preantral stage, whereas large follicles have already differentiated by 21 days of age [29-31]. FSH receptors are expressed in the preantral follicles of adult rats [30], and the follicles from adult animals are repeatedly exposed to cyclic changes in gonadotropins, estrogen, and progesterone. The effect of underlying differences in follicular environment on follicular development remains to be clarified.

In conclusion, the present study has demonstrated that activin A stimulates folliculogenesis of enclosed preantral follicles while, FSH has a synergistic effect with activin A. In addition, FSH showed folliculogenetic activity in the preantral follicles but combined action of activin A and FSH had a significant positive effect. Present study has shown that activin and FSH have a positive effect on follicle diameter and survival rate along with a significant increase in the percentage and number of matured oocytes and GVBD.

**ACKNOWLEDGEMENTS**

Financial supports from the Research and Clinical Center for Infertility, Yazd, Iran and Molecular Biochemistry Lab., Department of Biochemistry, University of Agriculture, Faisalabad, Pakistan are gratefully acknowledged. The authors are thankful to Prof. Abbas Aflatonian, Dr. Abdoli, Mr. Mehrdad Suleimani (Research and Clinical Center for Infertility, Yazd, Iran) and Mr. Abbas Baghi (Yazd Medical Institute of Biotechnology & Genetic Engineering, Science and Technology Park, Yazd, University of Medical Sciences, Yazd, Iran) for their kindest help during this work.

**REFERENCES**

10. McNatty, K.P., Heath, D.A., Lundy, T., Fidler, A.E., Quirke, L., O'Connell, A., Smith, P., Groome, N. and
Tisdall, D.J. (1999) Control of early ovarian follicular development. *J. Reprod. Fertil. Suppl.* 54: 3-16.


