Introduction

The key to fertility in a female are a pair of functional ovaries, fallopian tubes and the uterus. In the ovary, follicular development and ovulation are dependent on proliferation and differentiation of granulosa and theca cells which undergo steroidogenesis upon stimulation with gonadotropins and intraovarian cytokines (Richards 1994) . Granulosa cell of the ovary play a significant role in the production of sex steroid and myriad of growth factors which are involved in interaction with oocyte development through various signaling pathways (Maizels 2006). When these highly regulated processes go awry, infertility can occur. Infertility is defined as failure to achieve a clinical pregnancy after 12 months or more of regular unprotected relationship". Among the various proposed etiological factors, genetic, immunologic, endocrinologic and environmental disorders account for 50% of the patients; however, in remaining 50%, the cause remains unknown (idiopathic infertility). It is now thought that the idiopathic infertility might be due to lifestyle-environment interaction.

Because of the present life style, insulin resistance (IR) like condition has gained prevalence and is reported to be prevalent in 60-80% of women with polycystic ovarian syndrome (PCOS) (Diamanti-Kandarakis E 2012). Presence of insulin receptors in both stromal and follicular compartments of the human ovary and the demonstration of insulin's ability to stimulate steroidogenesis in ovarian cells *in vitro* has established the ovary as an important target organ for insulin action. In an insulin resistant ovary the classical pathway remains resistant to its metabolic activity, whereas the alternate pathway leading to production of androgens remains sensitive to insulin actions thus leading to hyperandrogenemia (Dunaif 1997). Insulin mediated increase in ovarian steroidogenesis is achieved through enhanced expression of genes such as steroidogenic acute regulatory protein (StAR), cholesterol side-chain cleavage cytochrome P450 (CYP11 A1), 3 β -hydroxysteroid dehydrogenase (3β-HSD), CYP19 A1 and 17β -hydroxysteroid dehydrogenase (17β-HSD) that are crucial for steroidogenic machinery. Hyperinsulinemia in IR alters the genes, enzymes, and proteins that are crucial for the steroidogenic machinery ultimately affecting follicle development and ovulation (Diamanti-Kandarakis 2008).

Environmental endocrine disruptors are known to cause female reproductive dysfunctions (Diamanti-Kandarakis et al., 2009). Cadmium (Cd) is an endocrine disruptor which is relatively dispersed in the environment mainly because of pollution. Cd, because of no known biological function, low rate of excretion from the body and long biological half-life accumulates over time in blood, kidney, liver and in the reproductive organs such as placenta, testis and ovaries. Previous studies in our lab with subclinical dose of 0.05mg/Kg b.w clearly demonstrated

inhibitory effects of Cd on hypothalamus pituitary ovarian axis (Pillai A 2005; Pillai 2005; Pillai 2002), key ovarian steroidogenic enzymes in ovary and granulosa cells (Gupta 2006; Nampoothiri *et al.* 2007; Nampoothiri LP 2008; Priya *et al.* 2004) and different developmental stages in rodents (Pillai *et al.* 2010). Apart from reproductive disorder, results of both human and animal studies suggest an association between Cd exposure, elevated blood glucose levels and the development of diabetes (Edwards and Prozialeck 2009).

To date studies have mainly focused on investigating the effect of Cd and IR individually on granulosa cells ultimately affecting fertility. It has been observed that Cd and IR are prevalent in today's life style. There is not a single study showing association between IR and Cd leading to infertility. In light of this, the present study was proposed to understand the etiopathogenesis of the combined effect of Cd and IR in rat and human granulosa cells with respect to altered cellular and molecular functions. This study was designed into two different aspects, wherein one focused on association between Cd and IR and other on understanding the changes in insulin and steroidogenic signaling in IR condition in human granulosa cells thereby developing intervention strategy by using some insulin-sensitizer bio-active compound. Combined effect of Cd and IR did alter the steroidogenic machinery of granulosa cells but the effect was not synergistic. Characterization of the insulin resistant condition at the molecular level would shed more light on the candidate molecules responsible for its pathogenesis. Interventions with the bio-active molecule would help in restoring the steroidogenic capacity of the granulosa cells thus formulating new therapy for infertility.

Specific objectives: Major Objectives of the present study are.

1: To study the effect of cadmium on cell death and expression of steroidogenic and major candidate genes in insulin resistant ovarian cells.

2: A. To study the effect of sub-clinical dose of cadmium on steroidogenesis in insulin resistant human granulosa cells.

B. To elucidate mechanism underlying insulin and steroidogenic signaling in insulin resistant human granulosa cells.

3: To identify insulin sensitizer bio-active compound to ameliorate insulin resistance and steroidogenic dysfunction in human granulosa cells.

Objective-1: To study the effect of cadmium on cell death and expression of steroidogenic and major candidate genes in insulin resistant ovarian cells.

Evidences state that, because of the change in lifestyle, there are increasing number of women with some degree of IR (Arrais 2006). Moreover, due to environmental issues, women are also exposed to toxicants such as Cd in their reproductive age (Diamanti-Kandarakis *et al.* 2008). As evident in present scenario, females are eventually exposed to both Cd and IR condition which may lead to altered reproductive performance and fertility-related problems. Because of their increased prevalence, an association between them could be responsible for idiopathic infertility which accounts for 50% of the total infertility cases. To prove this hypothesis, we performed an experiment wherein we made 4 groups: control, Cd, IR and IR+Cd. We exposed adult female *Charles Foster* rats with sub clinical dose of Cd (0.05mg/Kg b.w) for 15 days and with dexamethasone (3mg/Kg b.w) for 28 days for creating IR animal model both individually and in combination for studying the effect on estrus cyclicity, ovarian morphology, transcriptome and protein profiling of steroidogenesis. With treatment of 28 days, we demonstrated prolonged estrous cyclicity and insulin resistance condition in groups treated with dexamethasone alone and in combination with Cd. The current finding suggest that, sub clinical dose of Cd was insufficient to create IR condition, which is in concordance to earlier reports by Edward et al. 2010(Edwards and Prozialeck 2009). Further, abnormal histology of ovary, decrease in serum levels of estradiol and progesterone, amelioration in transcriptome and protein expression analysis of steroidogenic acute regulatory protein (StAR), cholesterol side-chain cleavage cytochrome P450 (CYP11A1), 3β-hydroxysteroid dehydrogenase (3β-HSD), 17β-hydroxysteroid dehydrogenase (17β-HSD) and cytochrome P450 aromatase (CYP19A1) along with decrease in enzyme activity and gonadotrophin receptors in groups co exposed with Cd and IR as compared to control and those exposed individually with Cd and IR (Belani *et al.* 2014).

In our above study we observed that Cd and IR together altered steroidogenesis. This effect could be primary due to decrease in granulosa cell numbers or secondary due to effect on hypothalamus and pituitary. In this context, we further aimed to reveal the combined effect of Cd and IR on granulosa cell death. Significant decrease in granulosa cell viability was observed in the individual and combined groups along with increase in protein expression of cleaved PARP-F2 and active caspase-3 confirming the apoptosis as the mode of cell death by AnnexinV-PI staining. No significant difference was observed between groups treated individually with IR or Cd when compared to the combined group. Apoptosis, is a physiological process to control cellular growth and is responsible for follicular atresia (Diamanti-Kandarakis *et al.* 2009). Increase in granulosa cell apoptosis as observed in present

study deprives oocytes from nutrients, survival factors and cell cycle proteins required for the achievement of meiotic competency of follicular oocytes prior to ovulation, ultimately leading to empty follicles, fewer and poor quality oocytes (Chaube *et al.* 2014). Also, at HPO axis, GnRH released from the hypothalamus induced the physiological release of FSH and LH in order to promote the development of the ovarian follicle and these sequential events are regulated through feed back mechanisms (Arrais 2006). In the present study significant decrease observed in mRNA expression of GnRH, CYP19A1, FSH-β and LH-β in combined and individually treated groups as compared to control group suggested that along with primary effect, secondary effect also played a major role in altering the steroidogenesis which was again in concordance with the literature. Overall results of this objective suggested that combined effect of IR and Cd lead to disturbances in steroidogenesis at cellular and molecular level and the effect might be both due to primary and secondary reasons.

Objective-2

A. To study the effect of sub-clinical dose of cadmium on steroidogenesis in insulin resistant human granulosa cells.

Alterations in steroidogenesis in insulin resistant rat granulosa cells treated with Cd inquisited us to study the sub-clinical effect of Cd in insulin resistant human granulosa cells *in vitro*. In this context, we standardized isolation protocol for human granulosa cells from IVF patients using method of Foldesi et al., 1998 and used this as a model system for our further studies (Foldesi *et al.* 1998). The characterized isolated cells were positive for steroidogenic markers StAR, CYP19A1, 17β-HSD, 3β-HSD, surface marker CD44 and mesenchymal marker vimentin fulfilling the requirements of a granulosa cells (Czernobilsky *et al.* 1985; Ohta *et al.* 1999).

To pursue studies relevant to insulin resistance condition associated with infertility, there was an urgent need for human insulin resistant granulosa cells. Hence, we next screened granulosa cells obtained from follicular fluid samples of PCOS patients and observed that 50% PCOS follicular fluid granulosa cells showed decreased IR, increased p-IRS-1(307) and increased PPAR-γ protein expression. As the study aimed to observe the combined effect of sub clinical Cd exposure along with IR condition on reproduction, a dose of 32µM Cd was standardized by MTT assay and the same dose was observed to be present in follicular fluid samples of cigarette smoking female patients (Paksy *et al.* 1997). The combined effect of insulin resistant granulosa cells with 32µM Cd for 24 hrs showed decrease in protein expression of StAR, CYP19A1, 17β-HSD and 3β-HSD along with decrease in progesterone and estradiol concentrations in the granulosa cell culture supernatant in combined and individually treated

group as compared to control. We further unraveled the reason behind decrease in human granulosa cell steroidogenesis and observed that the combined and individual effect of IR and Cd increased protein expression of cleaved PARP-F2, active caspase-3 indicating apoptosis as the mode of cell death which we further confirmed by showing positive ANNEXINV/PI staining.

Rat and human granulosa cells revealed that although the combined effect showed deleterious effect on granulosa cell steroidogenic machinery, it did not show significant reduction in the steroidogenesis as a whole when compare to the IR and Cd group individually indicating that life style and environmental conditions with respect to IR and Cd did not show any synergistic effect at cellular and molecular level and hence we further focused our study on understanding the mechanism involved in insulin resistant signaling in granulosa cell.

2B. **To elucidate mechanism underlying insulin and steroidogenic signaling in insulin resistant human granulosa cells.**

Insulin plays a role in normal follicular development of the ovary and hence in a variety of insulin resistant states ovarian dysfunctions are manifested (Poretsky *et al.* 1999). In insulin resistant condition the "central paradox" is that granulosa cells remains sensitive to insulin action to produce androgens in spite of systemic insulin resistant state; whereas classical target organs of insulin as well as ovary remain resistant to its metabolic activity(Mukherjee and Maitra 2010). Several reports also reveal cross talk between supra physiological doses of insulin via IGF-1R and gonadotroph signalling ultimately affecting expression of steroidogenic proteins and steroidogenesis in insulin resistant human luteinized granulosa cells (Mukherjee and Maitra 2010). In the present study, we would also like to explore alterations occurring in insulin and steroidogenic cascade in insulin resistance condition which might help in diagnosing insulin resistance at cellular level in IVF samples.

In view of understanding the mechanism of insulin action and steroidogenic pathway in insulin resistant human granulosa cells, we propose to analyse protein expression of IR-β, p-IRS(ser307), PI(3)K, p-AKT, PKCs and PPAR-γ , mRNA expression of IGF-1R, IGF-IIR, FSH-R and LH-R and mRNA and protein expression of StAR, CYP19A1, 3β-HSD and 17 β-HSD in 20 insulin resistant patients. Screening of 10 PCOS follicular fluid granulosa cells revealed significant down-regulation of IR-β and PI(3)K and up-regulation of p-IRS(ser307), p-AKT and PPAR-γ only in 50% of the samples. PPARγ has been shown to play important role in regulating ovarian follicle development, ovulation, oocyte maturation, and maintenance of the corpus lutem by enhancing or inhibiting steroidogenesis (Froment *et al.* 2006). Over expression of PPAR-γ gene in PCOS ovary and the presence of PPAR-γ1 binding sites in

differentially expressed genes suggest its role in pathogenesis of the syndrome. In a subgroup of PCOS women, IR appears to be related to excess serine phosphorylation of the IRS suggesting presence of serine/threonine kinase extrinsic to INSR or due to an inhibitor of a serine/threonine phosphatase accounting for hyperinsulinemia and hyperandrogenemia (Mukherjee and Maitra 2010). Up-regulation was observed in protein expression of p-AKT in granulosa cells of PCOS patients showing IR which was in line with the available human studies indicating that ovarian steroidogenesis involves AKT activation and that steroid metabolism is preserved in women with hyperinsulinemia and PCOS (Wu *et al.* 2012). Protein and mRNA expression of whole insulin signaling pathway, PKCs and MAPKs with more number of patients is under progress.

Objective-3: To identify insulin sensitizer bio-active compound to ameliorate insulin resistance and steroidogenic dysfunction in human granulosa cells.

Approximately 10- 15% of the couples are infertile. Decreasing the number of people affected by infertility has become a top priority for many health organizations. The life style factors such as age, physical exercise, psychological stress, cigarette smoking and sedentary life that impact fertility are modifiable and are considered to be the first-line of treatment (Diamanti-Kandarakis E 2012). Pharmacological intervention are considered as an adjunct to lifestyle modification (Sharma *et al.* 2013). Considering the role of insulin resistance in the interplay of metabolic and reproductive aberrations in infertility, insulin sensitizing therapy is expected to have beneficial effects.

To date metformin, rosiglitazone, pioglitazone and D-chiro-inositol have been effective in improving infertility related to insulin resistance in PCOS. Amongst these metformin is associated with improved clinical pregnancy but there are no evidence for improved live birth rates. Owing to the fact that metformin is also associated with a significantly higher incidence of gastrointestinal disturbances and decrease in Anti Mullerian Hormone levels its role in improving reproductive outcomes in women with insulin resistance appears to be limited (Tang *et al.* 2012).

In recent years, there has been renewed interest in the treatment of insulin resistance using herbal drugs, as World Health Organization (WHO) has recommended evaluation of the effectiveness of plants due to side effects of modern drugs (Baby Joseph 2011). In line with this our lab has also investigated effect of swertiamarin, a major compound found in *Enicostemma littorale* Blume in amelioration of insulin resistance condition in peripheral tissues. Swertiamarin has been observed to correct carbohydrate and fat metabolism by improving insulin sensitivity and by modulating PPAR-γ_2 in insulin dependent peripheral

tissues (Patel et al., 2013). Swertiamarin modulates adipogenesis and obesity related complications by interacting with major transcription factors.

In this context, insulin resistant human granulosa cells would be treated with swertiamarin and curcumin and protein expression of IR-β, p-IRS(ser307), PI(3)K, p-AKT, PKCs and PPAR-γ and mRNA expression of IGF-1R, IGF-IIR responsible for insulin signaling and mRNA expression of FSH-R and LH-R and mRNA and protein expression of StAR, CYP19A1, 3β-HSD and 17 β-HSD responsible for steroidogenic signaling would be analysed. Hence in the present study efforts would be made to ameliorate insulin resistance condition by the most potent bio-active molecule thus improving steroidogenic function and reducing the load of infertility.

Conclusion: Combined effect of insulin resistance condition and environmental endocrine disruptor Cd in granulosa cells of rats and humans altered steroidogenic capacity which was primary due to granulosa cell death by apoptosis and secondary due to alterations at hypothalamus and pituitary axis, however the effect was not observed to be synergistic. Signaling studies will help in unraveling the altered insulin and steroidogenic pathway in insulin resistance condition, which can be further exploited as diagnostic tool for screening of IR patients in IVF. The herbal insulin sensitizer will help in restoring the steroidogenic capacity thus helping in preventing infertility.

Reference:

Arrais, R. F. D., S. A. (2006). The hypothalamus-pituitary-ovary axis and type 1 diabetes mellitus: a mini review. *Hum Reprod* **21**, 327-37.

Belani, M., Purohit, N., Pillai, P., Gupta, S., and Gupta, S. (2014). Modulation of steroidogenic pathway in rat granulosa cells with subclinical Cd exposure and insulin resistance: an impact on female fertility. *Biomed Res Int* **2014**, 460251.

Chaube, S. K., Shrivastav, T. G., Tiwari, M., Prasad, S., Tripathi, A., and Pandey, A. K. (2014). Neem (Azadirachta indica L.) leaf extract deteriorates oocyte quality by inducing ROS-mediated apoptosis in mammals. *Springerplus* **3**, 464.

Czernobilsky, B., Moll, R., Levy, R., and Franke, W. W. (1985). Co-expression of cytokeratin and vimentin filaments in mesothelial, granulosa and rete ovarii cells of the human ovary. *Eur J Cell Biol* **37**, 175-90.

Diamanti-Kandarakis, E. (2008). Polycystic ovarian syndrome: pathophysiology, molecular aspects and clinical implications. *Expert Rev Mol Med* **10**, e3.

Diamanti-Kandarakis, E., Argyrakopoulou, G., Economou, F., Kandaraki, E., and Koutsilieris, M. (2008). Defects in insulin signaling pathways in ovarian steroidogenesis and other tissues in polycystic ovary syndrome (PCOS). *J Steroid Biochem Mol Biol* **109**, 242-6.

Diamanti-Kandarakis, E., Bourguignon, J. P., Giudice, L. C., Hauser, R., Prins, G. S., Soto, A. M., Zoeller, R. T., and Gore, A. C. (2009). Endocrine-disrupting chemicals: an Endocrine Society scientific statement. *Endocr Rev* **30**, 293-342.

Diamanti-Kandarakis E, C. C., Marinakis E. (2012). Phenotypes and enviromental factors: their influence in PCOS. *Curr Pharm Des* **18**, 270-82.

Dunaif, A. (1997). Insulin resistance and the polycystic ovary syndrome: mechanism and implications for pathogenesis. *Endocr Rev* **18**, 774-800.

Edwards, J. R., and Prozialeck, W. C. (2009). Cadmium, diabetes and chronic kidney disease. *Toxicol Appl Pharmacol* **238**, 289-93.

Foldesi, I., Breckwoldt, M., and Neulen, J. (1998). Oestradiol production by luteinized human granulosa cells: evidence of the stimulatory action of recombinant human follicle stimulating hormone. *Hum Reprod* **13**, 1455-60.

Froment, P., Gizard, F., Defever, D., Staels, B., Dupont, J., and Monget, P. (2006). Peroxisome proliferator-activated receptors in reproductive tissues: from gametogenesis to parturition. *J Endocrinol* **189**, 199-209.

Gupta, L. P. N. a. S. (2006). Simultaneous effect of lead and cadmium on granulosa cells: a cellular model for ovarian toxicity. *Reproductive Toxicology* **21**, 179-185.

Maizels, M. H.-D. a. E. T. (2006). FSH signaling pathways in immature granulosa cells that regulate target gene expression: Branching out from protein kinase A. *Cell Signal.* **18**, 1351-1359.

Mukherjee, S., and Maitra, A. (2010). Molecular & genetic factors contributing to insulin resistance in polycystic ovary syndrome. *Indian J Med Res* **131**, 743-60.

Nampoothiri, L. P., Agarwal, A., and Gupta, S. (2007). Effect of co-exposure to lead and cadmium on antioxidant status in rat ovarian granulose cells. *Arch Toxicol* **81**, 145-50.

Nampoothiri LP, G. S. (2008). Biochemical effects of gestational coexposure to lead and cadmium on reproductive performance, placenta, and ovary. *J Biochem Mol Toxicol.* **22**, 337-44.

Ohta, N., Saito, H., Kuzumaki, T., Takahashi, T., Ito, M. M., Saito, T., Nakahara, K., and Hiroi, M. (1999). Expression of CD44 in human cumulus and mural granulosa cells of individual patients in in-vitro fertilization programmes. *Mol Hum Reprod* **5**, 22-8.

Paksy, K., Rajczy, K., Forgacs, Z., Lazar, P., Bernard, A., Gati, I., and Kaali, G. S. (1997). Effect of cadmium on morphology and steroidogenesis of cultured human ovarian granulosa cells. *J Appl Toxicol* **17**, 321-7.

Pillai A, G. S. (2005). Effect of gestational and lactational exposure to lead and/or cadmium on reproductive performance and hepatic oestradiol metabolising enzymes. *Toxicol Lett* **15**, 179-86.

Pillai, A. G., S. (2005). Antioxidant enzyme activity and lipid peroxidation in liver of female rats co-exposed to lead and cadmium: effects of vitamin E and Mn2+. *Free Radic Res* **39**, 707-12.

Pillai, A. L. P., P. N.Gupta, S. (2002). Effects of combined exposure to lead and cadmium on pituitary membrane of female rats. *Arch Toxicol* **76**, 671-5.

Pillai, P., Pandya, C., Gupta, S., and Gupta, S. (2010). Biochemical and molecular effects of gestational and lactational coexposure to lead and cadmium on ovarian steroidogenesis are associated with oxidative stress in F1 generation rats. *J Biochem Mol Toxicol* **24**, 384-94.

Poretsky, L., Cataldo, N. A., Rosenwaks, Z., and Giudice, L. C. (1999). The insulin-related ovarian regulatory system in health and disease. *Endocr Rev* **20**, 535-82.

Priya, P. N., Pillai, A., and Gupta, S. (2004). Effect of simultaneous exposure to lead and cadmium on gonadotropin binding and steroidogenesis on granulosa cells: an in vitro study. *Indian J Exp Biol* **42**, 143-8.

Richards, J. (1994). Hormonal control of gene expression in the ovary. *Endocr Rev* **15**, 725-751.

Sharma, R., Biedenharn, K. R., Fedor, J. M., and Agarwal, A. (2013). Lifestyle factors and reproductive health: taking control of your fertility. *Reprod Biol Endocrinol* **11**, 66.

Tang, T., Lord, J. M., Norman, R. J., Yasmin, E., and Balen, A. H. (2012). Insulin-sensitising drugs (metformin, rosiglitazone, pioglitazone, D-chiro-inositol) for women with polycystic ovary syndrome, oligo amenorrhoea and subfertility. *Cochrane Database Syst Rev* **5**, CD003053.

Wu, S., Divall, S., Wondisford, F., and Wolfe, A. (2012). Reproductive tissues maintain insulin sensitivity in diet-induced obesity. *Diabetes* **61**, 114-23.

Publication:

1. **Muskaan Belani**, Nupur Purohit, Prakash Pillai, Sharad Gupta, and Sarita Gupta. "Modulation of Steroidogenic Pathway in Rat Granulosa Cells with Subclinical Cd Exposure and Insulin Resistance: An Impact on Female Fertility," *BioMed Research International*. Volume 2014, Article ID 460251, 13 pages.
2. Nidheesh Dadheech, Abhay Srivastava, **Muskaan Belani**, Sharad Gupta, R.R. Bhonde, Anand S Srivastava and Sarita Gupta, "Basal Expression of Pluripotency Associated Genes

Can Contribute to Stemness Property and Differentiation Potential", Stem Cells Dev. 2013, Jun 15;22(12):1802-17.

Abstract Published and Poster Presented:

1. **Muskaan Belani**, Nupur Purohit , Sarita Gupta "**Exposure of environmental endocrine disruptor cadmium on insulin resistant luteinized granulose cells: effect on ovarian morphology, cell viability and transcriptome machinery**" in Gujarat Science Congress 2012.
2. **Muskaan Belani**, Nupur Purohit , Sarita Gupta "Influence of "insulin resistance" and endocrine disruptor 'cadmium' on luteinized granulosa cells". Abstract Book, National Conference & 30th Annual Symposium of the Society for Reproductive Biology and Comparative Endocrinology (SRBCE) on Novel Aspects and Emerging Trends in Reproduction and Endocrinology, 2012.
3. Prakash Pillai, **Muskaan Belani**, Sarita Gupta. Biochemical and molecular mechanism of cellular toxicity by lead and cadmium in luteinized granulosa cells: *In vitro* exposure studies. Annual Symposium of the Society for Reproductive Biology and Comparative Endocrinology (SRBCE) on Novel Aspects and Emerging Trends in Reproduction and Endocrinology, 2010.

Achievements:

1. Received CSIR-SRF Research Fellowship Award for three years– 2010-2013.
2. Awarded second prize in poster presentation at National conference on – Diabetes and its complications - Search for prevention and cure. 6-7th sep, 2013. **Muskaan Belani**, Nupur Purohit, Nidhi Gupta, Sarita Gupta. **Influence of insulin resistance and environmental endocrine disruptor cadmium on cell death in luteinized granulosa cells.**

Date: 8th October, 2014 **Signature of the candidate**

Muskaan Anil Belani

(Prof. Sarita Gupta)

Guide

Head **Dean**

Biochemistry Department **Faculty of science**