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Research Proposal

Mammalian ovary is the key female reproductive organ that consists of various follicles containing different cells like theca, granulosa, cumulus and an ovum. These follicles undergo various developmental stages like follicular growth, ovulation and leutinization under the control of gonadotrophin (LH:FSH) surge. Development of ovarian follicles and the breakdown of the follicular wall to release the mature oocyte during ovulation as well as the formation of corpus luteum from luteinizing follicular cells; all involve extensive tissue remodeling process which are possibly controlled by different proteinase systems such as matrix metalloproteinases (such as MMP2, MMP9, MMP-MT1) as well as hyaluronan and hyaladherins. In most mammals including human, soon after LH surge or hCG treatment, synthesis of hyaluronan in follicular fluid via the expression of HAS2 in granulosa and cumulus cells, and its organization into an ECM along with various hyaladherins (like TSG6, HABP1, PTX-3, CD44 etc) results into COCs expansion/maturation.

In the present study, we developed superovulated which showed a number of periovulatory mature follicles. We also developed two anovulatory models. In the first model rats were treated with RU486 (PCO model). The histology of the ovary showed that the normal follicles were lacking and numerous follicular cysts were formed and multilayered granulosa are seen in this anovulatory model. Whereas another model was developed by treating rat with indomethacin. Histology of this treated ovary showed intraovarian follicular rupture resulting in, cells and fluid movement within ovary. We found that the levels of HA, TSG6, MMP-MT1 and COX2 are upregulated during follicular maturation and ovulation in super-ovulated model while such levels are comparatively altered/upregulated in PCO and Indomethacin treated anovulatory rats. Further superovulated model showed a gradual increment in HABP1 expression till ovulation (12 hrs) and then it represses during leutinization (beyond 12 hrs) while in case of PCO and IM treated models, the levels of HABP1 are reduced during follicular development and ovulation. Also ovarian

MMP2 (pro- and active- form) expression and activities are significantly elevated during ovulation in anovulatory models as compared to superovulated rat. Such decrease in HABP1 levels and elevated MMP2 activities in anovulatory models might be due to vastly upregulated concurrent expression of MMP-MT1 that can degrade HABP1 and cleaves MMP2 to activate it. This is also supported by the co-localization of HABP1, MMP-MTI and MMP2 in follicular fluid of ovarian tissue in all rat models that gives the clear evidence for possible interaction of these proteins.

We observed that LH+FSH induced invitro COCs expansion remained uneffected by RU486 and indomethacin while anti-HABP1 antibody treatment along with LH+FSH completely blocked COC expansion, indicating that gonadotropin surge triggered COCs expansion is mediated by HABP1. The expression levels of some of the important molecular factors like hyaluronan, COX2, TSG6, MMP-MT1; all are found to be altered in anovulatory models during follicular development and ovulation. Moreover all these genes along with HAS2 are already reported to be controlled by transcription factor NfkB; thus we have explored the related signaling pathway in *invitro* and *invivo* models, by implying thymoquinone (TQ); a recently identified inhibitor of Nf-kB activation/translocation. In KK1 cells NfkB activation/translocation is suppressed by TQ treatment when given alone as well as along with other drugs. Moreover expression levels of HA, TSG6, HABP1, MMP-MT1, active-MMP2 are found to be normalized in TQ treated anovulatory models (*PCO model showed more prominent effect). In addition TQ treated PCO ovary is restored about 70-80% normal ovulatory follicles while only 30-40% such effect is seen in TQ+IM treated rat.

Thus in the present study, we have demonstrated, the reversion of anovulatory condition to normal ovulatory process by targeting Nf-kB pathway and thus related gene products. On the basis of all above observations, we propose for the first time that the intervention of Nf-kB activation/translocation and related gene expression using thymoquinone could be a promising safe herbal remedy for related ovarian pathophysiologies such as polycystic ovary. This opens up new insights into the mechanism underlying the regulation of acute/chronic inflammation and may form a basis for management of related disorders, as a future prospect.