Summary

In majority of the fishes reproduction is seasonal. Because of *Anabas* ovary is synchronous in nature , that is, at a time we found only one stage of the oocyte from four different stages of follicle (previtellogenic, vitellogenic, post vitellogenic and post- GVBD). The reproductive processes of *Anabas*, which include gonadal recrudescence, vitellogenic event and final maturation occur in a specific time of the year. The master of the endocrine activity is initiated by the decapeptide hormone, gonadotropin-releasing hormone (GnRH) which is secreted from brain and eventually regulate the reproductive events in all vertebrates. Chronologically GnRH then stimulate, LH and FSH acts on two cell layer in pulsatile manner which respectively induce both the theca and granulosa cell layer to synthesize 17α- hydroxy progesterone and 17β-estradiol respectively, two cell two gonadotroph hypothesis.

In addition to steroids and gonadotropins, ovarian function is controlled by several other factors in teleosts. Recent research has demonstrated that cytokines are crucial for teleost fish reproduction. Inflammation and fish ovulation are both similar in some ways. Fish contains all major cytokine families, which is now generally accepted as a result of earlier research and reviews. By secreting locally, they can control ovarian processes. Pro-inflammatory cytokines like IL-1β, TNF-α, and IL-6, as well as their various paralogues, are found in the fish ovary of the majority of species. Prostaglandins (PGs) and the pro-inflammatory cytokine tumor necrosis factor (TNF-α) may control important stages of the mammalian ovulatory process, according to studies.

In the current work, the levels of ovarian IL-6, TNF-α , and IL-10 were measured in both in vivo and in vitro experiments at different stages of *A. testudineus* maturation. In the course of its inquiry, the current study discovered that only the post-GVBD stage has the potential to secrete IL-6, suggesting that IL-6 may be involved in the ovulatory processes of *A. testudineus*. Gonadal IL-6 secretions were higher in fish treated with hCG at the post-vitellogenic and pre-ovulatory (post-GVBD) stages; this could be explained by the oocytes becoming more sensitive to gonadotropins. After hCG treatments, there were increased levels of IL-6 at the Pt-GVBD stage, and these higher

levels persisted. IL-6 may activate other signalling pathways that result in oocyte shrinkage since *A. testudineus* cannot reproduce unless it is combined with a gravid male. The inhibition of hCG-induced gene expression when there was treatment with TAPI-I in an in vitro investigation, validates the regulatory function of gonadotropin-mediated TNF-α secretion. We observed that the secretion of TNF-α was high only in the vitellogeneic follicle when treated with hCG but the impact of hCG did not change the secretion of TNF-α in post vitellogenic follicle indicates their involvement in the maturational phase in *Anabas* oocytes but not during the time of the ovulation. Investigation was take interesting turn, when our in vivo study suggested that the secretion of IL-10 in untreated fish was gradually increase pre-vitellogenic follicle to post-vitellogenic follicle in *Anabas*. The same pattern of secretion was also observed when the fish treated with hCG. The in vitro study also confirmed the result of in vivo experiment. Whenever the follicles were treated with TAPI-1 secretion level of IL-10 was lower and also down regulates the mRNA transcripts in oocyte. The interesting thing observed in this experiment is that secretion pattern of TNF-α and IL-10 was similar in post vitellogenic and post-GVBD follicle. This result establish the fact that the production of IL-10 was directly controlled by the secretion of TNF-α. The kinetic study approach also establish the fact their is an involvement of both of the cytokines in different stages of the oocyte of *A. testudineus*. So, we can clearly say that the pro- inflammatory (IL-6) and anti-inflammatory cytokine (IL-10) play a pivotal role in the reproductive processes of the teleost.

More closer look for the involvement of IL-6 and IL-10 reveal that external IL-6 dosen't involve during the process of oocyte maturation rather it induce the rate of ovulation in dose dependent manner. Rate of ovulation is highest when treated with 2ng/ml dose. Where as in case of IL-10, rIL-10 neither involved in the process of oocyte maturation nor it can triggered the ovulation process single handedly. So, it was clear that during the time of ovulation IL-6 directly take part to conduct the process. The investigation also established when treated with hCG and TNF-α, but arrest the ovulation when treated with TAPI-1. Our experiment showed that IL-6 is one of the

primary cytokines influencing the breakdown of follicular walls and oocyte release by increasing the MMP activity during IL-6-induced in vitro ovulation. The zymography result which shows the activation of MMP clear out the fact. Furthermore, it increases the *pgf2,* which is one of the main physiological indicators of ovulation. The transcription level expression analysis of the ovulatory genes (eg. *ptgs2, cpla2*, and *mif* in this study) also supports the involvement of IL-6 in ovulation; all of these genes are markedly up-regulated in response to IL-6 treatment but down-regulated in response to TAPI-1. Furthermore, the fact that both *mmp-2/9* were up-regulated in response to IL-6 demonstrated that IL-6 functions as a crucial regulator during the ovulation process. Another study that shows *il-1β* is upregulated in response to IL-6 which can be also the cause of LH induced ovulation. In contrary, the relative abundance of other pro-inflammatory cytokines like *nfκβ*, *tnf*-α and *il-6* were all down regulated in post-vitellogenic follicle when treated with rIL-10 as compare to that of hCG which is generally a positive regulator of those cytokines. Most specific findings in this investigation was its interaction with IL-6. When post-vitellogenic follicle was treated with rIL-10 it down regulate the expression of *il-6* while those oocyte treated with rIL-6 the expression of *il-10*.

In the advance segment of our study we tried to unveil the interaction between IL-6 and IL-10 with steroid production in the different stages of *Anabas* follicles. The role of IL-6 on steroid production in different doses establish the fact that IL-6 does not change the level of E2 in vitellogenic and post vitellogenic follicle while it changes production of T in higher doses follicles were at vitellogenic and post vitellogenic stages. On the other hand, no significant correlation has been found between the IL-10 (in any doses) and steroid production in any stages of follicle. Establish the fact that IL-10 alone cannot make any significant differences during the time of the oocyte maturation and ovulation.

Next question remains that from where they secret. To find out the secretory area of IL-6 and IL-10 in the oocyte gives us an interesting fact that IL-6 was secreted from granulosa (not theca) cells. It

is likely that TNF-α plays a role in granulosa cells production of IL-6, which in turn causes apoptosis and ovulation. From where we stand their was not much research on the cellular origin of IL-10 in fish, our findings suggest that both the theca and the granulosa cells are capable of producing it. The fact that TNF-α controls the production of IL-10 could support our assertion.

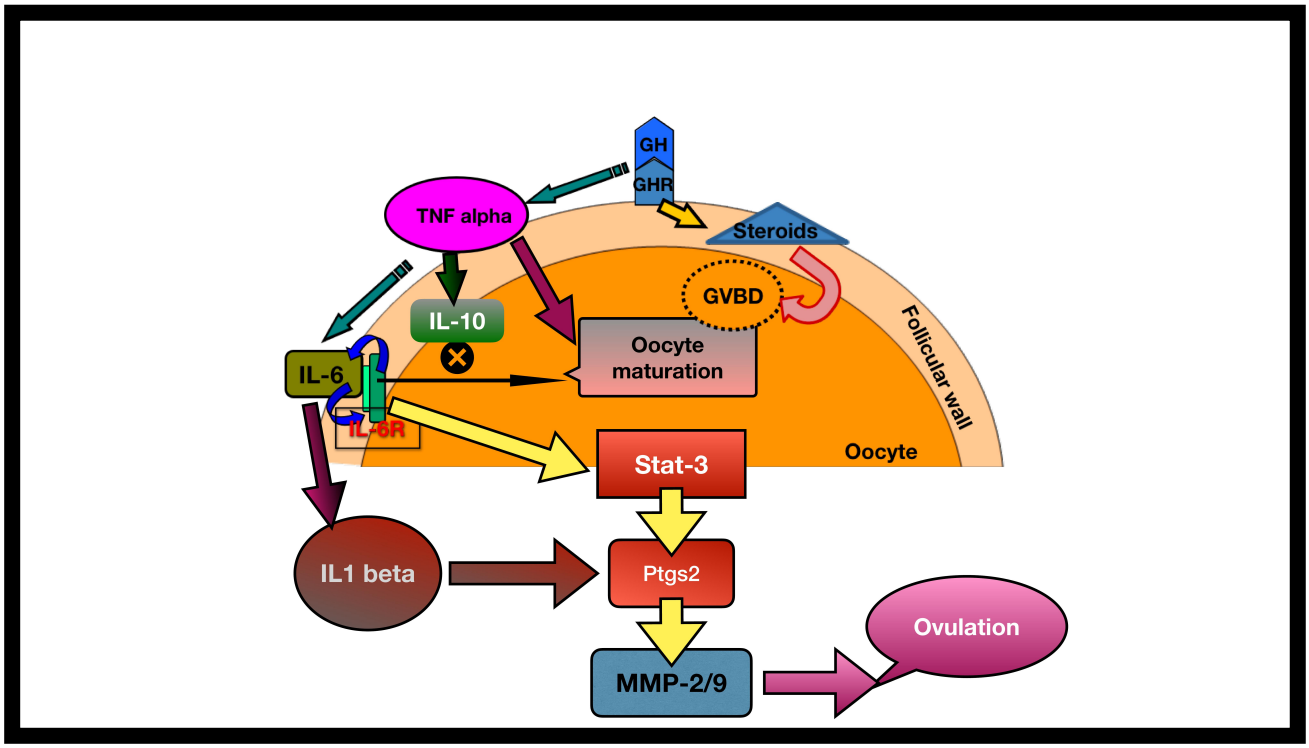
Now coming to the last part of our study, investigation in cellular level signalling transduction is the most decorated and establish platform to understand how they work. In our study, Western blot data analysis reveals that during the ovulation period, IL-6 upregulates the stat-3 receptor, through which they act. This is confirmed when TAPI subsequently blocks the expression of stat-3. From this study, we can also infer that stat-3 may function as a key regulator for activating MMP2 and facilitating ovulation.

The up regulation of IL-6 receptors in post vitellogenic follicle by IL-6 itself (auto regulation) but not by the gonadotropin explain the pivotal role of IL-6 during the time of the ovulation in *Anabas*. The eventual mechanism then controlled by the MMPs which may be activated by the stat-3 and ptgs2 tells us the fact that the ovulation process in fish is all interdependent between the gonadotropin-steroids-paracrine factors in that particular microenvironment of that oocytes. The up regulation of IL-10 receptors when treated with gonadotropin and TNF-α establish an argument whether IL-10 is important for the reproductive processes or not. From our investigation it was clear that the dependency of Il-10 release with TNF-α and its constant presence in vitellogenic and post vitellogenic processes establish the scenario that IL-10 may take part to maintain the homeostasis of other cytokines to ensure proper environment for oocyte to grow and eventually matured.

So, in conclusion IL-6 and IL-10 play a very crucial role during the time of reproductive processes of teleost. IL-6, secreted from granulosa cells of the oocyte played a vital role during the time of ovulation by activating MMP via activating stat-3. This pathway can be direct or they can also induce the production of IL-1β which can induce ovulation by LH surge. IL-10 directly is not

responsible for maturation or ovulation of teleost oocyte. Rather its production is controlled by TNF-α and it may play the role of maintaining homeostasis by control the pro inflammatory cytokine synthesis in ovary. As a whole IL-6 and IL-10 both are crucial to maintain a healthy oocyte in *Anabas*.

Where the story stands now



A schematic representation of the interaction between the different cytokine and paracrine factors during the reproductive processes of a female teleost, Anabas testudineus.