CHAPTER 2

Influence of administration of FGF-2 and antiFGF-2 on the nucleic acids and protein profiles of the regenerating tail of wall lizard *Hemidactylus flaviviridis*

INTRODUCTION

The process of epimorphic regeneration is highly complex. It includes several physiological processes like the healing of wound, matrix reorganization, apoptosis of damaged cells, angiogenesis, neurogenesis, cell proliferation, differentiation etc. However, a noticeable feature of the early stages of epimorphic regeneration is the hypertrophy of the blastemal cells leading to the rapid growth of the regenerate During amphibian limb regeneration, after the formation of wound epithelium (WE), the dedifferentiated cells undergo a series of hasty cell divisions to accumulate a mass of pleuripotent blastemal cells and further bring about the restoration of the lost limb Several experiments performed on the epimorphic regeneration in amphibians have established that FGF-2 is involved in the proliferative activities during early events of epimorphic regeneration (Gardiner and Bryant, 1996, Mullen *et al.*, 1996, Zhang *et al*, 2000, Ferretti *et al.*, 2001). Further the activity of FGF-2 is up-regulated in the regenerating spinal cord of newts, and it is also found in a subset of blastemal cells and chondroblasts, and in the basal epidermal layer (Ferretti *et al.*, 2001). Moreover, FGF-2 has also been shown to be expressed in a multitude of proliferating cells For example, basic fibroblast growth factor (FGF-2) has been shown to influence the survival, and proliferation of a variety of cell types in the nervous system of rats (Cavanagh *et al.*, 1997)

Further, the role of FGF-2 in promoting proliferation of different types of cells has been cited by several investigators (Gospodarowicz, 1978, O'Keefe, 1988, Dignass *et al*, 1994). FGF-2 acts as a potent mitogen for many central nervous system (CNS) populations (Cattaneo and McKay, 1990, Lillien and Cepko, 1992, Kilpatrick and Bartlett, 1993, DeHamer *et al.*, 1994, Ray and Gage, 1994, Vicario-Abejón *et al.*, 1995) Besides, *in vitro* studies have shown that high molecular weight forms (HMW) of FGF-2, which are primarily localized in the nucleus, are known to modulate cell proliferation (Bugler *et al*, 1991, Florkiewicz *et al.*, 1991; Mignatti *et al*, 1991, Quarto *et al*, 1991, Renko *et al.*, 1991; Bikfalvi *et al*, 1995) In addition, FGF-2 is also known to induce endothelial cell proliferation, migration, and angiogenesis *in vitro* (Basilico *et al*, 1992). Similarly, Schwartz *et al.*, (1993) have demonstrated that FGF-2

stimulates smooth muscle cell proliferation and is a potent mitogen for human stromal cells and delays their senescence (Oliver *et al*, 1990). Also, neural precursors isolated from adult rat brain are induced to proliferate and to differentiate by FGF-2 (Richards *et al*, 1992) However, the process of epimorphic regeneration requires a population of stem cells that can later be differentiated to remold the lost appendage.

The recruitment of stem cells can be achieved either by aggregation of already existing stem cells, or by the dedifferentiation of the adult stump cells In amphibians and possibly also in lizards the stem cells are recruited by the process of dedifferentiation (Kumar *et al.,* 2000) The process of dedifferentiation requires the entry of quiescent adult cells into the mitotic phase Several factors are thought to play role in this process, with FGF-2 being one such growth factor Studies carried out by Korr *et al.,* (1992) have shown that in cell culture FGF-2 leads to a single re-entry of G0 rat astroglial cells into the mitotic cycle Thereafter, FGF-2 does not affect the mode of proliferation Moreover, several investigators have indicated that FGF-2 is released during and as a result of cell injury in rats (Clarke *et al.*, 1993; Ku *et al.*, 1995; Calara *et al.*, 1996, Kaye *et al*, 1996) Further, fibroblast growth factor-2 (FGF-2) promotes cardiac myocyte proliferation and has been detected in extracellular as well as cytoplasmic and nuclear compartments The involvement of FGF-2 in cell proliferation was further authenticated by Liu *et al*, (1997) who reported anti-FGF-2 labeling in interphase, metaphase and telophase nuclei in chicken myocytes, thus indicating that FGF-2 is active participant during cell cycle In addition, FGF-2 induces DNA synthesis in murine aortic endothelial cells (Ferretti *et al.*, 2001) Moreover, it stimulates proliferation and migration of several intestinal epithelial cell lines (Dignas *et al*, 1994, Bikfalvi *et al*, 1997, Burgess, 1998, Jones *et al.*, 1999, Werner, 1998)

In many tumor cells and cell lines, FGF-2 is known to play role in transformation and proliferation of various cell types (Neufeld *et al*, 1988, Sasada *et al*, 1988; Quarto *et al.*, 1991; Nguyen *et al.*, 1994; Bikfalvi *et al.*, 1997). According to Ware and Post (1999) FGF-2 causes proliferation and subsequent rearrangement of endothelial cells on the extracellular matrix in humans *In vitro*, FGF-2 is mitogenic for immature oligodendrocytes in rats and promotes their survival, while blocking oligodendrocyte maturation (Saneto and DeVillis, 1985, McKinnon *et al.*, 1990, Goddard *et al.*, 1998). Furthermore, FGF-2 is highly expressed in bovine adrenal tissues and is one of the most potent mitogens for cultured adrenal cells (Gospodarowicz *et al.*, 1986, Penhoat *et al.*, 1988, Mesiano *et al*, 1991; Feige *et al*, 1998). Further, according to Mesiano *et al*, (1991) FGF-2 and Insulin like growth factor-II (IGF-II) show cooperative mitogenic effect in human fetal adrenal cells Similarly, interactions between FGF-2 and the IGF system have been described in proliferation in various cell models including muscle, chroanoffic, hypothalamic, and neuroblastoma cells in rats (Pons

et al, 1992, Frodin *et al.*, 1994). Thus, FGF-2 is present in many tumor cells and cell lines and has been shown to be involved in transformation and proliferation of various cell types (Neufeld *et al.*, 1988; Sasada *et al*, 1988, Quarto *et al*, 1991, Nguyen *et al.*, 1994, Bikfalvi *et al.*, 1997). Hence, this mitogenic nature of FGF-2 could induce rapid proliferation of blastemal cells during the process of regeneration, which increases the length of the regenerate However, after certain growth of the regenerate, the proliferative activities are confined to the leading edge and the process of differentiation takes over in the region closer to the stump tissue.

Several *in vitro* studies have established that FGF-2 regulates the process of differentiation Like, FGF-2 stimulates differentiation of several intestinal epithelial cell lines (Dignas *et al.*, 1994, Bikfalvi *et al.*, 1997, Burgess, 1998, Werner, 1998; Jones *et al.*, 1999) Also, neural precursors isolated from adult rat brain are induced to proliferate and to differentiate by FGF-2 (Richards *et al.*, 1992) Further, differentiation of a variety of cell types in the nervous system of rats has been shown to be influenced by FGF-2 (Cavanagh *et al.*, 1997) In addition, differentiation of normal human melanocytes is dependent on FGF-2 (Halaban *et al.*, 1992). Globus *et al.*, (1988) have also documented that the proliferation and differentiation of bovine osteoblasts are stimulated by FGF-2 while it is an exogenous regulator of smooth muscle cell migration and proliferation in mammals (Blaes and Allera, 1997) Besides, the proliferation and differentiation of mesodermal tissues, such as fibroblasts and endothelial cells, as well as neuroectodermal cells in humans is stimulated by FGF-2 (Montesano *et al.*, 1986, Baird and Walicke, 1989, Bennett and Schultz, 1993, Bennett and Schultz 1993, Bhora *et al.*, 1995; Gibran *et al.*, 1995) These reports suggest that FGF-2 is a modulator of differentiation in a number of cell types Moreover, FGF-2 has also been shown to be localized in differentiating muscle of amphibian tail regenerate (Ferretti *et al.*, 2001)

Thus, all these studies indicate that FGF-2 is a potent mitogenic molecule for a variety of cells and it is also a key modulator of amphibian regeneration. Further, FGF-2 is a positive regulator of differentiation for some type of cells, while it negatively regulates differentiation in others Since FGF-2 has a significant influence on tail regeneration in gekkonid lizard, as has been shown by our studies (Chapter I), it was of immense interest to know whether FGF-2 is involved in the proliferation and/or redifferentiation of the blastemal cells to replace the lost tail. Hence, it was thought worthwhile to study the influence of FGF-2 on the nucleic acids and protein profiles in the regenerate at different stages of tail regeneration in *Hemidactylus flaviviridis*

MATERIAL AND METHODS

Healthy wall lizards, *Hemidactylus flaviviridis,* of similar physical characteristics were selected and acclimated for a week in the animal house A total of seventy-two animals were used and they were divided into six groups of twelve animals each Animals in each group were treated as follows:

Group I The animals received IP injection of 0 6% saline
Group II· Received IP injection of FGF-2 at a dosage of 25μg/kg b.wt
Group III: The animals of this group were given IP injection of antiFGF-2, 25mg/kg b wt
Group IV Animals were administered 0 6% saline *in loco*
Group V Animals were administered FGF-2 *in loco* at a dose of 12.5 μg/kg b wt
Group VI. Animals were subjected to antiFGF-2 *in loco*, 12.5 mg/kg b.wt

All the drugs were prepared in 0 6% physiological saline, fresh before use Each of the drugs was administered at a dosage of 0 05ml/kg b wt intraperitoneally while the *in loco* injection was given at a dosage of 0 025ml/kg b wt. The drugs were administered every alternate day The administration of drugs started four days before autotomy and was continued till the animals reached differentiation stage

From each group, six animals, which attained the early blastema (BL) stage on the same day, were sacrificed. The nucleic acids and protein content in the regenerate was estimated Further, from the remaining animals, the animals which reached differentiation (DF) stage on the same day were sacrificed and the protein and nucleic acids were quantified in the regenerate

Histofluorescent localization of nucleic acids: The regenerates from the above groups were excised after hypothermic anesthesia at three stages *viz* WE stage, BL stage and DF stage, and transferred to cryostat microtome at –25^{0}C Tissues were fixed in OCT compound (Tissue Tek-II) and sectioned at 10μm The sections were stained with acridine orange and observed for nucleic acids localization under fluorescence microscope (DMRB, Leica) with epi-illumination with filter settings of 440 nm excitation filter and 510 nm barrier filter

STATISTICAL ANALYSIS: The data was analyzed by One Way ANOVA with Duncan's Multiple Range Test The values are expressed as Mean ± SE A 'p' value of 0.05 or less was considered statistically significant

RESULTS

Treatment with FGF-2:

BL stage: The group of animals treated with FGF-2 intraperitoneally showed an elevated level of DNA in the regenerate at the BL stage, compared to control lizards (Table 2 1; Figures 2 11, 2 12 and 2 1). Similar effects were observed in lizards treated *in loco* The RNA levels were also significantly higher ($p \leq 0.01$) in the FGF-2 treated lizards, both *in loco* and IP, compared to control lizards (Figure 2 3) However, the protein content was not altered much in FGF-2 treated animals, as compared to control animals (Figure 2 5) There was a significant boost ($p \leq 0$ 01) in DNA/RNA ratio in the blastema of the animals treated with FGF-2 (Figure 2.7) The RNA/Protein ratio also showed significant ($p \leq 0$ 01) hike in animals treated with FGF-2 (Figure 2.9)

DF stage: The regenerates in both the experimental groups, IP and *in loco* FGF-2 treatments maintained significantly higher ($p \leq 0$ 01) levels of DNA during early DF phase (Table 2 2; Figures 2.2, 2 13) The RNA as well as protein levels were significantly higher ($p \leq 0.01$) in experimental groups (Figure 2 4 and 2.5) Though, IP treatment with FGF-2 showed a significant higher ($p \leq 0.05$) DNA/RNA ratio, this ratio was not significantly different from control animals in *in loco* treated animals (Figure 2 8). However, the RNA/Protein ratio was significantly lowered ($p \leq 0$ 01) in the treated lizards during differentiation phase of tail regeneration (Figure 2.10)

Treatment with antiFGF-2:

BL stage: The DNA content in the antiFGF-2 treated groups (both IP and *in loco* treated animals) was significantly lowered ($p \leq 0$ 01) when compared to control group, at BL stage of tail regeneration in *Hemidactylus flaviviridis* (Table 2 1, Figures 2 1, 2 11 and 2 12) Though the RNA levels were significantly lowered ($p \leq 0$ 01) in the animals treated with antiFGF-2, the DNA/RNA ratio was comparatively higher in IP ($p \leq 0$ 05) and *in* loco ($p \leq 0$ 01) treated animals as against that observed in control lizards (Figures 2 3, 2 7) The animals treated with antiFGF-2 showed a significantly lower ($p \leq 0$ 01) RNA/Protein ratio at the BL stage, as is evident from the lower protein content when compared to that of controls (Figures 2 5, 2 9)

DF stage: There was a significant decrease ($p \leq 0.01$) in the DNA content in the antiFGF-2 treated lizards as compared to control animals (Table 2 2, Figure 2 2, 2 13) The animals treated with antiFGF-2 showed significantly lowered ($p \leq 0$ 01) RNA and protein levels in the regenerates (Figures 2 4, 2.6). The DNA/RNA ratio was not significantly altered in the treated animals as compared to control animals, but RNA/protein ratio showed a significant decrease ($p \leq 0.01$) (Figures 2. 8, 2.10).

DISCUSSION

In the present study, the mitogenic role of FGF-2 was assessed by studying nucleic acid profile. The administration of extraneous FGF-2 was found to increase the levels of DNA in the regenerate, at BL and DF stages of tail regeneration On the contrary, the animals treated with antiFGF-2 showed decreased levels of DNA at both the stages The high levels of DNA in the regenerates of FGF-2 treated animals might have resulted from the high rate of replication of DNA in the dividing cells of the regenerate Thus, FGF-2 might have been involved directly or indirectly in the proliferation of blastemal cells in the regenerates of the lizards The administration of FGF-2 was found to increase the proliferation of cells even in the wound epithelium (WE) stage. This was observed by the histoflourescence studies. The WE of FGF-2 treated animals showed higher yellow fluorescence (DNA localization) as compared to control animals. Besides, the thickness of the WE was found to be more in FGF-2 treated animals, which might be due to increase in the number of cells, resulting from the amplified cell proliferation However, the yellow fluorescence was found to be appreciably lowered in the WE of antiFGF-2 treated animals The cellular organization of the WE of antiFGF-2 treated animals was found to be non-uniform, as compared to control animals These observations signified that FGF-2 could be involved in the formation of a functional WE Similar to this, several investigators have shown that treatment with FGF-2 enhances cellularity during healing of the wound by increasing the number of cells (Kuhn *et al.,* 2001, Greenhalgh *et al* , 1990), hence a more uniform and compact WE was observed in FGF-2 treated animals During epimorphic regeneration, however, the formation of WE is followed by the development of blastema

The formation of a functional blastema requires the reentry of the quiescent cells into active cycles of cell proliferation. The present study showed that treatment with FGF-2 accelerated the formation of blastema Thus, FGF-2 might be pushing quiescent cells into active cycles of cell proliferation as Zeitler *et al* , (1997) have reported that FGF-2 induces the progression of the cell cycle from G0/G1 to S phase in endothelial cells. Moreover, in cultures of glial cells, FGF-2 stimulates mature cells to re-enter the cell cycle (astrocytes—Korr *et al.*, 1992,

oligodendrocytes — Fressinaud *et al.*, 1993; Schwann cells — Peulve *et al.*, 1994) In the present study, the regenerates of the lizards treated with FGF-2, both intraperitoneally and *in loco*, showed an increase in the DNA levels Thus, FGF-2 might be stimulating the synthesis of DNA in the blastemal cells in lizards. Moreover, several reports have confirmed that the fibroblast growth factors are potent inducers of DNA synthesis in a multitude of cells (Lathrop *et al*, 1985; Olwin *et al*, 1986, Imamura *et al.*, 1990; Kan *et al*, 1993, Vainikka *et al*, 1994; Wang *et al*, 1994, Wiedlocha *et al*, 1994). According to Liu *et al.*, (1997) the staining of chick embryo with anti-FGF-2 showed that chick FGF-2 or immunologically related protein(s) not only increase in DNA-synthesizing nuclei but they may play a role in subsequent stages of mitosis and cytokinesis In addition, FGF-2 is known to stimulate mRNA, DNA, and protein synthesis in fibroblasts and epithelial cells also (Gospodarowicz *et al*, 1990, Lindner *et al*, 1991, Rapraeger *et al*, 1991, Bennett and Schultz, 1993, Alberts *et al*, 1994, Hoppenreijs *et al*, 1994) Besides, *in vitro* studies have shown that FGF-2 activates MCM3 (minichromosome maintenance deficient 3), a factor essential for DNA replication licensing activity, and can be produced by blastemal cells themselves, indicating an autocrine action (Wang *et al*, 1997). These reports along with the findings of present study suggested that FGF-2 might be playing a key role in the initiation of blastema growth, as has been observed by Giampaoli *et al*, (2003)

The histoflourescence studies also supported the observations that FGF-2 increased the DNA content in the regenerates at the BL and DF stages The regenerates at the BL stage showed higher yellow fluorescence in FGF-2 treated animals while, results were opposite for antiFGF-2 treated animals Similarly, at DF stage too the regenerates of FGF-2 treated animals showed higher yellow fluorescence. Conversely, blocking the endogenously produced FGF-2 by administering antiFGF-2, showed a definite impairment of the proliferative activities during the early stages of tail regeneration. The DNA levels were significantly decreased in the regenerates of the animals treated with antiFGF-2 in the present study Similar results were also reported by Lindner *et al*, (1991), who demonstrated that addition of FGF-2 significantly increased medial smooth muscle cell proliferation when administered after injury of the rat carotid artery, and opined that medial smooth muscle cell proliferation could be significantly inhibited by neutralizing antibodies to FGF-2 Apart from the synthesis of DNA, the proliferating blastemal cells also transcribe RNA and synthesize new proteins to meet the demands of the rapidly dividing cells.

In the current study, the RNA levels were higher in regenerates in FGF-2 treated lizards at both the BL and DF stages as compared to that of control animals, but the transcriptional activities were found to be lowered in the regenerates of FGF-2 treated animals at the BL stage as evidenced by an elevated DNA to RNA ratio Similarly, the process of transcription

was found to be significantly lowered in the animals treated with FGF-2 intraperitoneally at the DF stage, while the animals treated with FGF-2 *in loco* showed comparable transcription with that of control animals. There are several reports which suggest that transcription is inhibited by FGF-2 For example, Carreras *et al.,* (2001) has shown that basic fibroblast growth factor (FGF-2) decreases elastin gene transcription in confluent rat lung fibroblasts. Treatment of astrocytes with basic fibroblast growth factor (FGF-2) produces a dramatic change from a polygonal to a stellate morphology, and results in a significant decrease in glial fibrillary acidic protein (GFAP) mRNA and protein (Reilly *et al.,* 1998). Similarly, FGF-2 decreases osteonectin transcripts in a dose- and time-dependent manner and this regulation is independent of the mitogenic effect of FGF-2 (Delany and Canalis, 1998) Further, treatment with antiFGF-2 showed a decrease in the RNA levels at both the BL and DF stages In the current study the animals treated with antiFGF-2 showed a lower rate of transcriptional activities as is evident from the high DNA/RNA ratio at the BL stage, while at DF stage there was no significant difference in the transcriptional activities between the control and experimental animals From these results it could be opined that FGF-2 did not necessarily play any explicit role in the transcriptional activities, as has been shown by the increased DNA/RNA ratio even in the antiFGF-2 treated animals. Thus, there might be some other factors and/or signals that might be triggering the process of transcription However, the elevated levels of RNA in the FGF-2 treated animals as compared to control animals could be accredited to the higher amount of DNA being accumulated owing to the higher rate of replication Likewise, the reduced levels of RNA observed in the regenerates of the antiFGF-2 treated animals might be as a result of lower DNA levels as compared to control animals, though the transcriptional activities were higher in the antiFGF-2 treated animals at the BL stage Similar results were obtained in the histofluorescence studies for RNA localization The regenerates of FGF-2 treated animals showed higher flame red fluorescence (RNA localization) at both the BL and DF stages, while those treated with antiFGF-2 showed opposite results, with lower fluorescence than control animals However, other investigators have shown that FGF-2 stimulates mRNA synthesis in fibroblasts and epithelial cells in rats (Gospodarowicz *et al.*, 1990, Rapraeger *et al.*, 1991; Bennett and Schultz, 1993, Alberts *et al* , 1994, Hoppenreijs *et al* , 1994; Wang *et al* , 1997), while the results of the current study showed that FGF-2 might not be playing any role in the process of RNA synthesis in the regenerates Furthermore, the synthesis of RNA is followed by the translation of mRNA into proteins in the regenerates

The translational activities were found to be lower in lizards treated with FGF-2 at the BL stage, but were increased as the lizards reached DF stage This might be probably because FGF-2 could be inhibiting the translation of mRNAs at the earlier stages of tail regeneration On the other hand, in the animals treated with antiFGF-2 there was a significant increase in

the process of translation at the BL stage and as the lizards reached the DF stage, the translational activities were elevated in FGF-2 as well as antiFGF-2 treated lizards From these results it might be concluded that administration of FGF-2 inhibited the translation process during early stages of tail regeneration while it did not play any role in the protein synthesis during the DF stage Moreover, during DF stage of tail regeneration in lizards, the synthesis of new proteins might be required more by the differentiating tissues, which include muscles, cartilage, neurons etc, than the proliferating cells Thus, the mitogenic role of FGF-2 appeared to vane during DF stage, as has been shown elsewhere (Chapter I) Further, FGF-2 is an inhibitor of differentiation, as it inhibits myoblast differentiation by suppressing MyoD and myogenin (Kruzhkova *et al.*, 2000), hence the observed decrease in the rate of translation in FGF-2 treated animals at BL stage could be an indicator of inhibition of differentiation of the regenerate. Moreover, analysis of MM14 mouse myoblasts demonstrates that terminal differentiation is repressed by pure preparation of basic fibroblast growth factor (FGF) (Clegg *et al.,* 1987) and FGF repression occurs only during the G1 phase of the cell cycle by a mechanism that appears to be independent of ongoing cell proliferation Thus it is evident from the above observations that FGF-2 might be present during initial events tail regeneration, while later on it might have been down regulated as the cells change their activities from proliferation to synthesis. Hence, in the present study, though there was an increase in the length of the regenerate due to rapid cell divisions in the FGF-2 treated animals, the process of differentiation was slightly altered as compared to control animals This was also evident from the histoflourescence studies. Thus, the results indicated that FGF-2 might be needed only during early stages of tail regeneration in *Hemidactylus flaviviridis*, while later stages were found to be independent of the presence of FGF-2 These results imply that FGF-2 has an important role in tail regeneration in gekkonid lizard and is likely to be involved both in proliferation and differentiation of tail tissues

In summary, it could be argued that FGF-2 act to augment the mitotic activity as evidenced by increased DNA levels in the regenerates, while at the same time it also significantly increased the levels of RNA However, the animals treated with FGF-2 showed significantly lower transcriptional and translational activities at the BL stage, while during the DF stage the synthetic activity was found to increase in the FGF-2 treated animals The current notion was further consolidated by the results of antiFGF-2 treated animals. However, the exact pathway by which FGF-2 exerts its mitogenic influence needs to be further evaluated

TABLE 2 1 The nucleic acids and protein content in the regenerates in the experimental and control groups in *Hemidactylus flaviviridis*, at the BL stage.

Treatment	Nucleic acids and protein levels in Regenerate		
	DNA (µg/100mg protein)	RNA (µg/100mg protein)	PROTEIN (mg/100mg tissue)
IP Control	0 486 ± 0 004[@]	0 776 ± 0 004	1 040 ± 0.022
IP FGF-2	0 870 ± 0 005**↑	0 908 ± 0 007**↑	1 032 ± 0 018
IP antiFGF-2	0 275 ± 0.009**↓	0 323 ± 0 010**↓	0 987 ± 0 008**↓
IL Control	0 515 ± 0 006	0 784 ± 0 010	1 263 ± 0 015
IL FGF-2	0 876 ± 0 010**↑	0 902 ± 0 011**↑	1 024 ± 0 024
IL antiFGF-2	0 270 ± 0 011**↓	0 278 ± 0 010**↓	0 985 ± 0 008**↓

Treatment	Nucleic acids and protein levels in Regenerate	
	DNA / RNA	RNA / PROTEIN
IP Control	0 627 ± 0 008	0 747 ± 0 017
IP FGF-2	0 958 ± 0 009**↑	0 881 ± 0 012**↑
IP antiFGF-2	0 858 ± 0 051*↑	0 327 ± 0 008**↓
IL Control	0 657 ± 0 002	0 621 ± 0 007
IL FGF-2	0 972 ± 0 021**↑	0 883 ± 0 023**↑
IL antiFGF-2	0 979 ± 0 056**↑	0 282 ± 0 01**↓

[@] Values are expressed as Mean ± SE, * p≤0 05, ** p≤0 01

TABLE 2.2: The levels of nucleic acids and protein in the regenerates of the experimental and control lizards at DF stage.

Treatment	Nucleic acids and protein levels in Regenerate		
	DNA (μg/100mg protein)	RNA (μg/100mg protein)	PROTEIN (mg/100mg tissue)
IP Control	0.956 ± 0.019[@]	0.396 ± 0.004	1.058 ± 0.019
IP FGF-2	1.235 ± 0.04**↑	0.470 ± 0.006**↑	1.880 ± 0.023**↑
IP antiFGF-2	0.449 ± 0.009**↓	0.211 ± 0.004**↓	0.869 ± 0.014**↓
IL Control	0.896 ± 0.004	0.417 ± 0.004	0.994 ± 0.006
IL FGF-2	1.221 ± 0.030**↑	0.443 ± 0.006**↑	1.789 ± 0.041**↑
IL antiFGF-2	0.456 ± 0.009**↓	0.224 ± 0.005**↓	0.837 ± 0.013**↓

Treatment	Nucleic acids and protein levels in Regenerate	
	DNA / RNA	RNA / PROTEIN
IP Control	2.419 ± 0.066	0.374 ± 0.009
IP FGF-2	2.630 ± 0.084*↑	0.263 ± 0.008**↓
IP antiFGF-2	2.128 ± 0.07	0.243 ± 0.008**↓
IL Control	2.149 ± 0.021	0.420 ± 0.004
IL FGF-2	2.762 ± 0.087	0.247 ± 0.003**↓
IL antiFGF-2	2.041 ± 0.077	0.268 ± 0.008**↓

[@] Values are expressed as Mean ± SE, * $p \leq 0.05$, ** $p \leq 0.01$

Figure 2 1 DNA content in Regenerate at BL stage

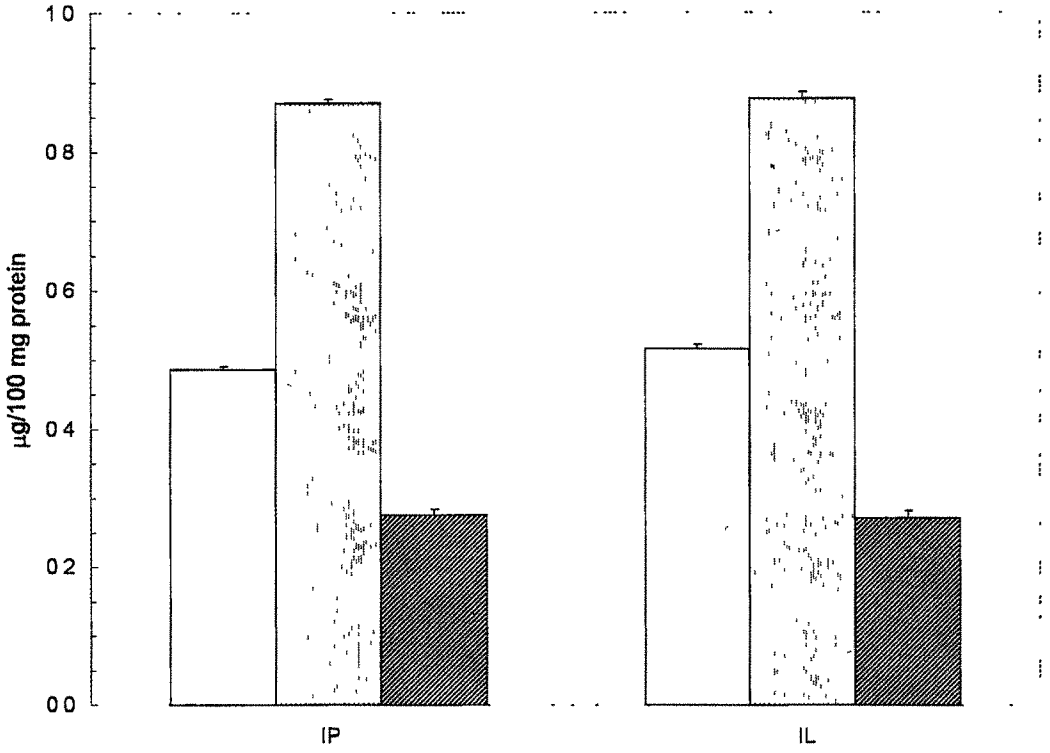

Figure 2 2 DNA content in Regenerate at DF stage

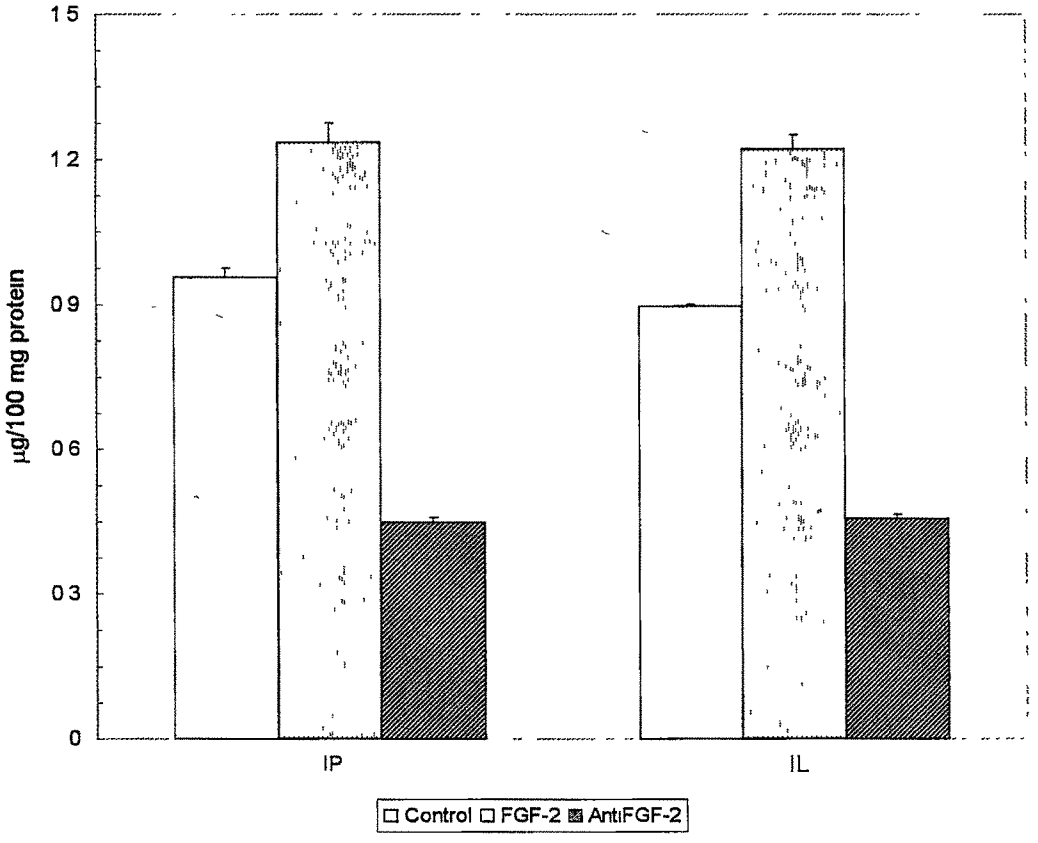

Figure 2 3 RNA content in Regenerate at BL stage

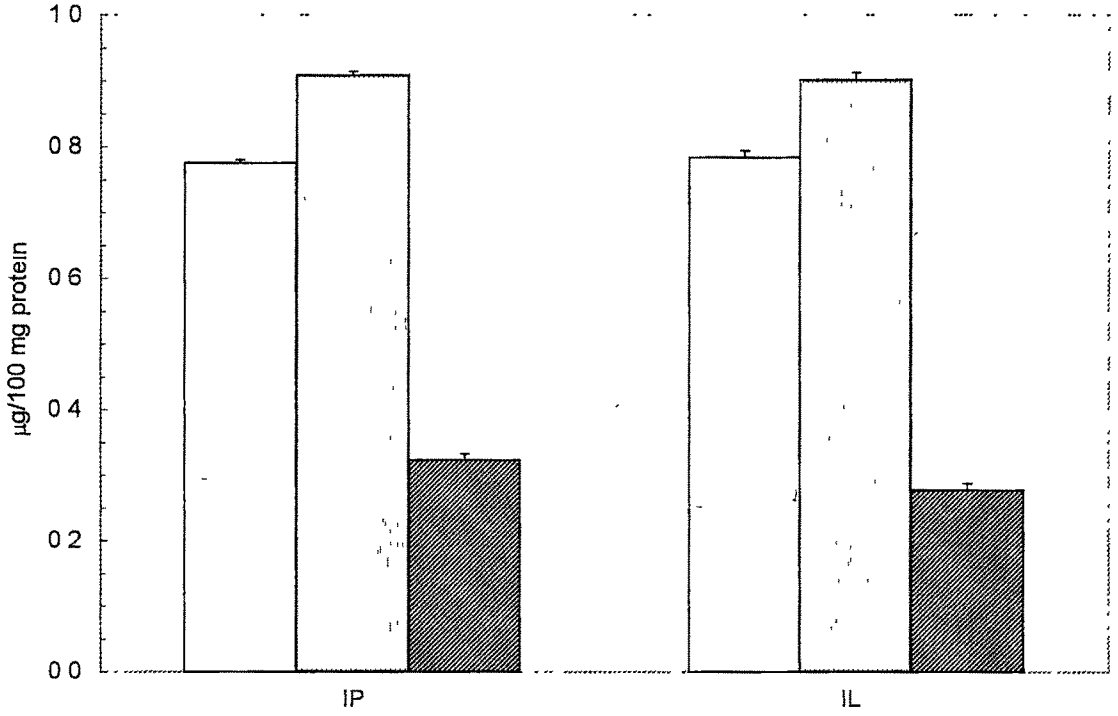

Figure 2 4 RNA content in Regenerate at DF stage

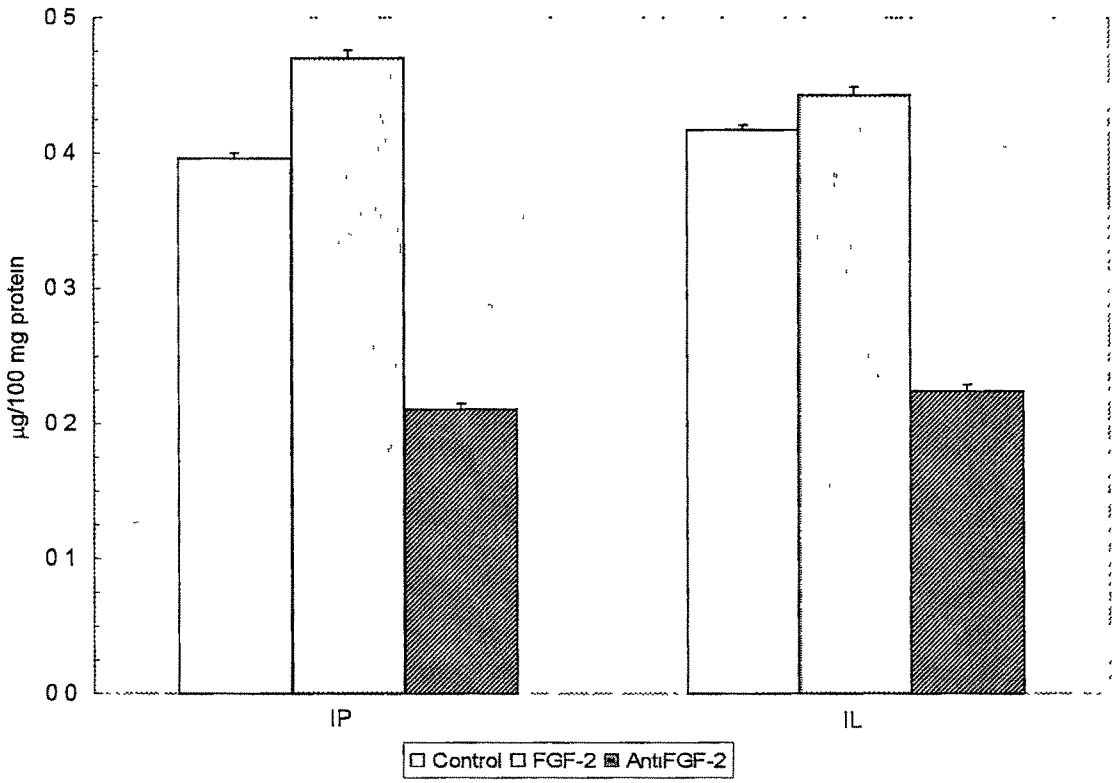

Figure 2 5 Protein content in Regenerate at BL stage

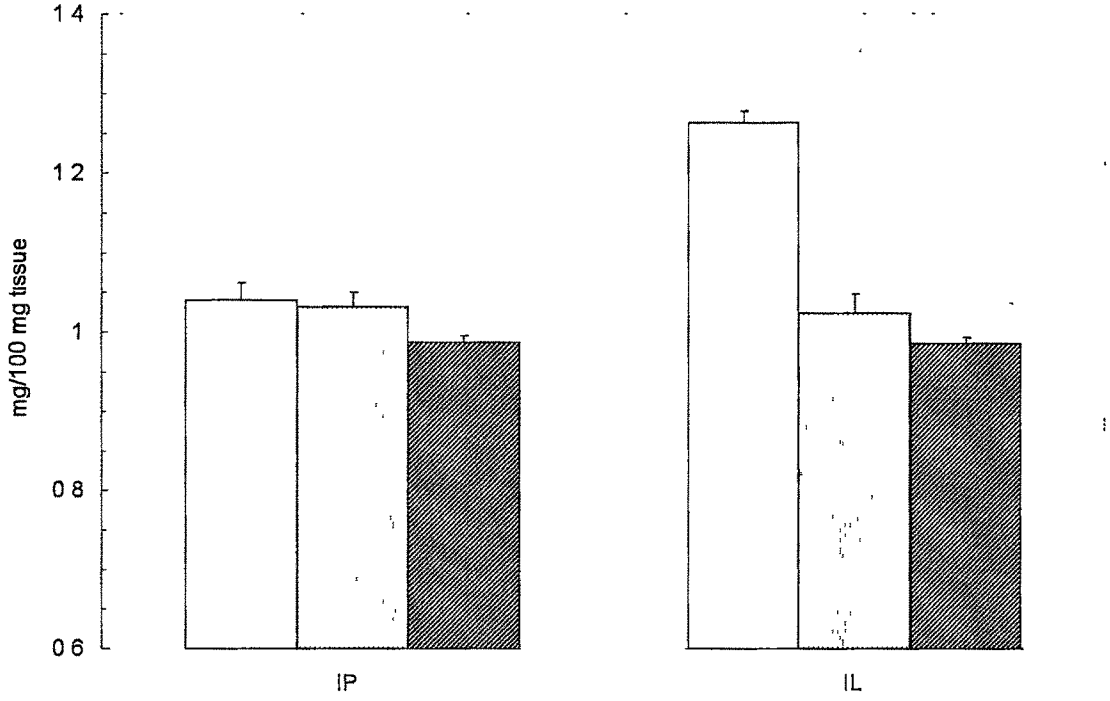

Figure 2 6 Protein content in Regenerate at DF stage

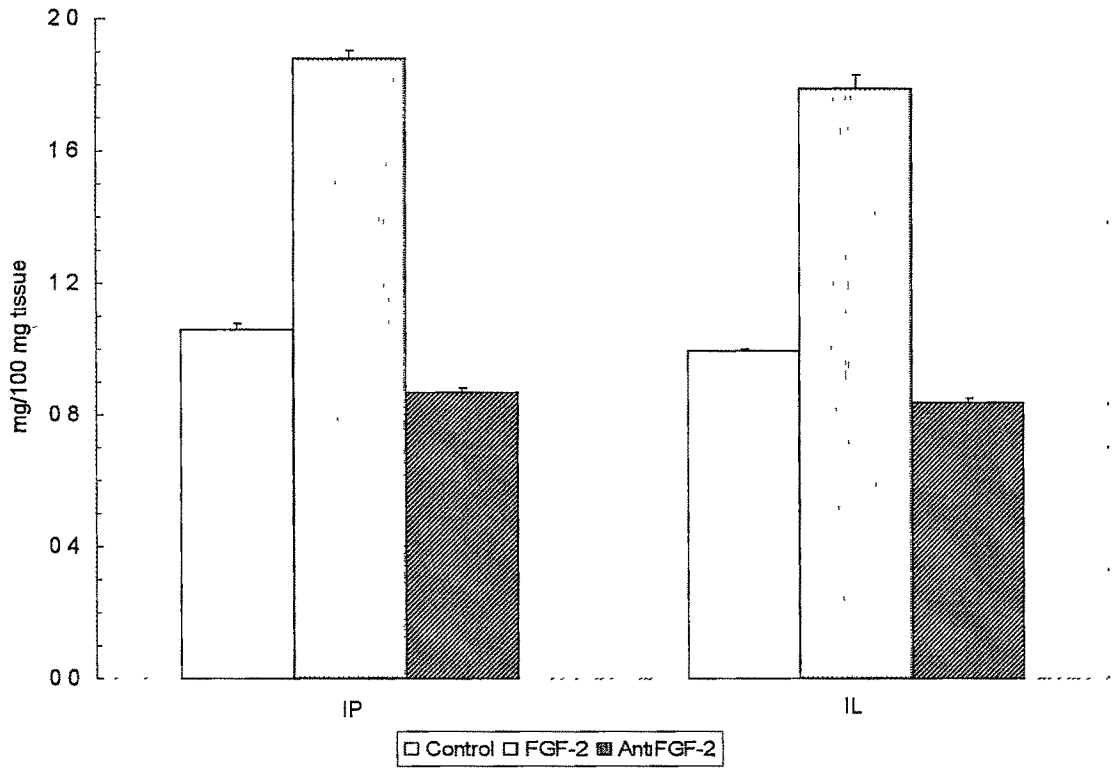

Figure 2 7 DNA/RNA ratio in the Regenerate at BL stage

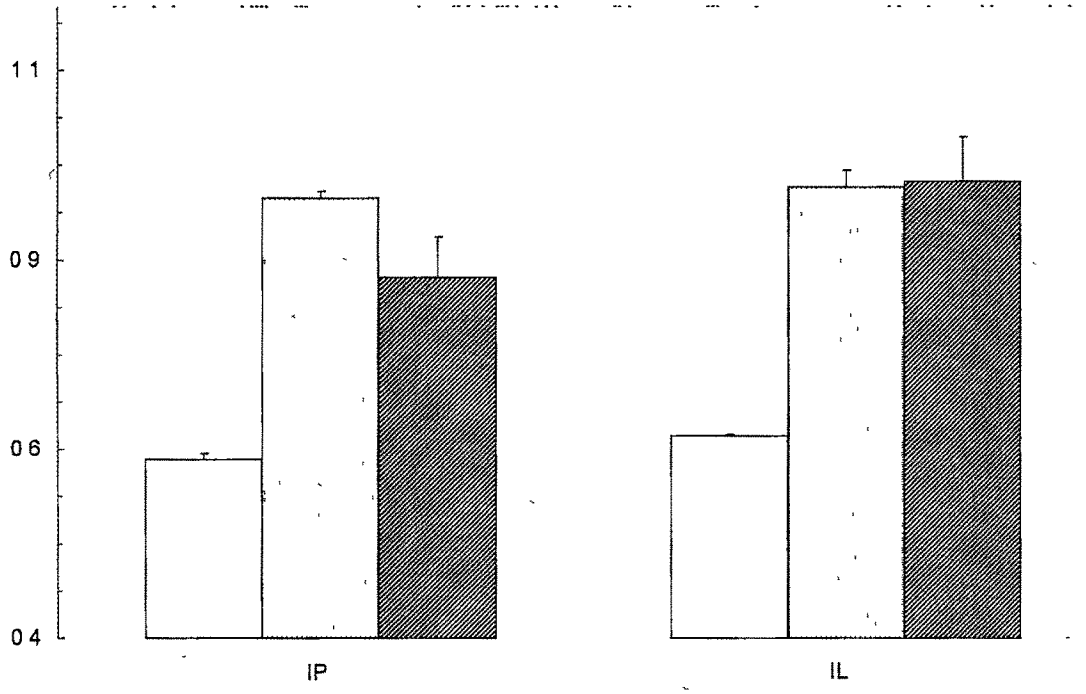

Figure 2 8 DNA/RNA ratio in the Regenerate at DF stage

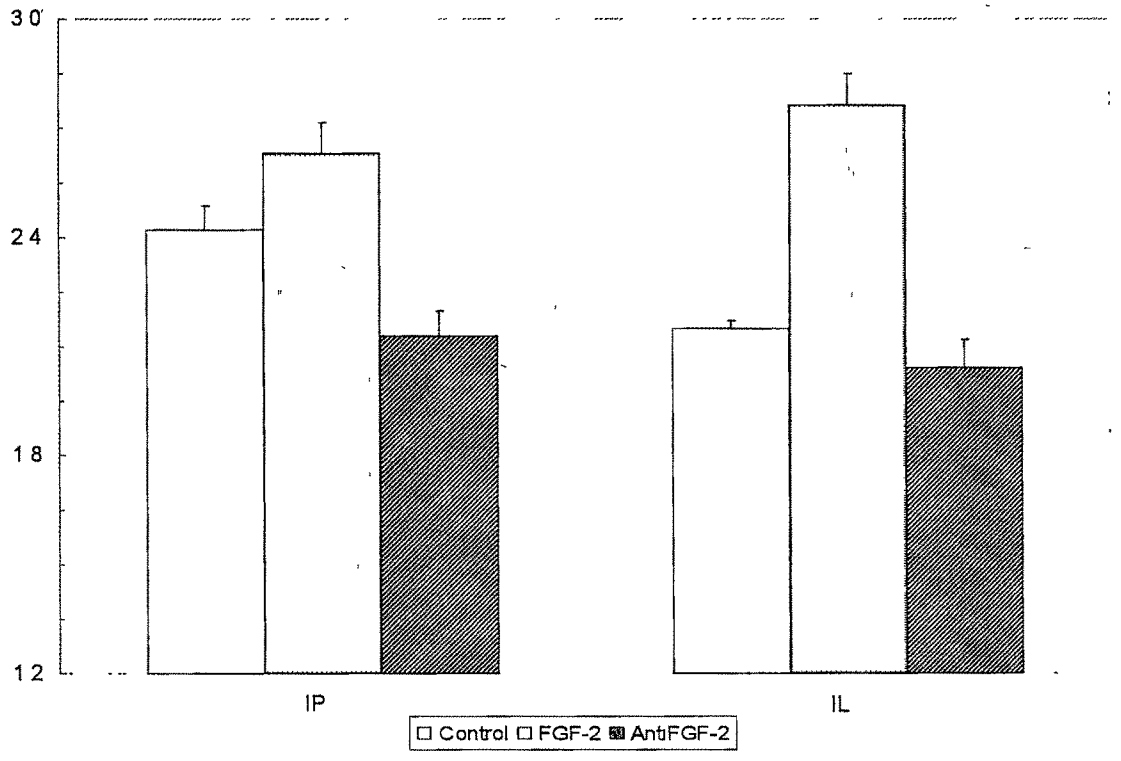

Figure 2 9 RNA/Protein ratio in the Regenerate at BL stage

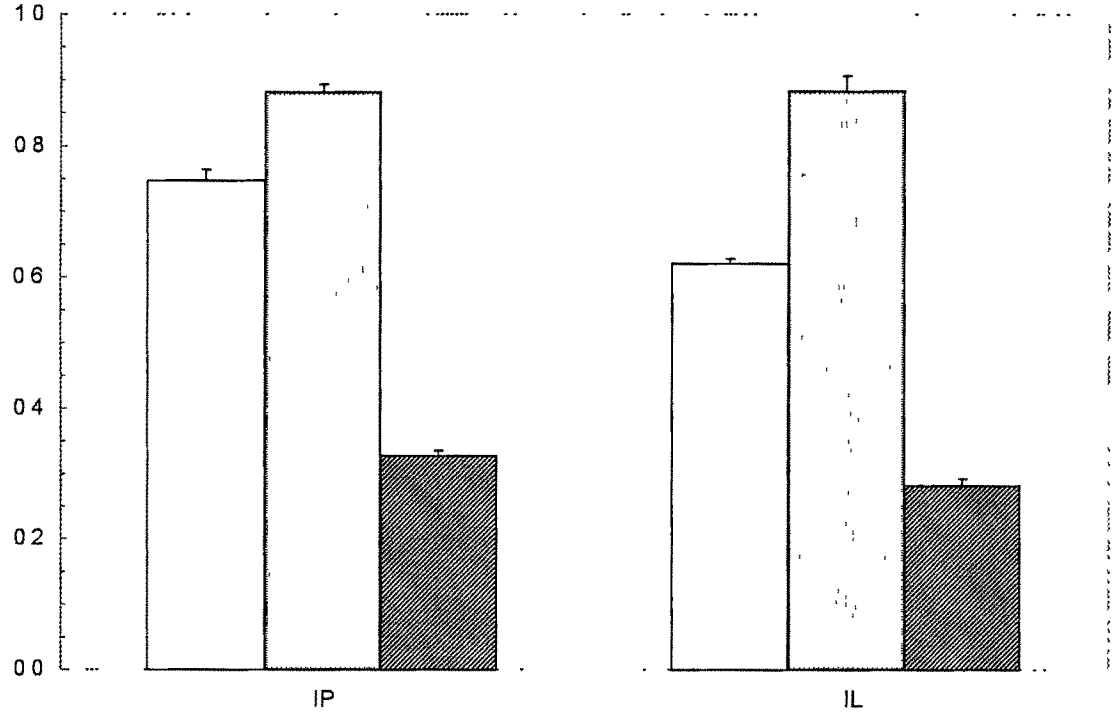

Figure 2 10 RNA/Protein ratio in the Regenerate at DF stage

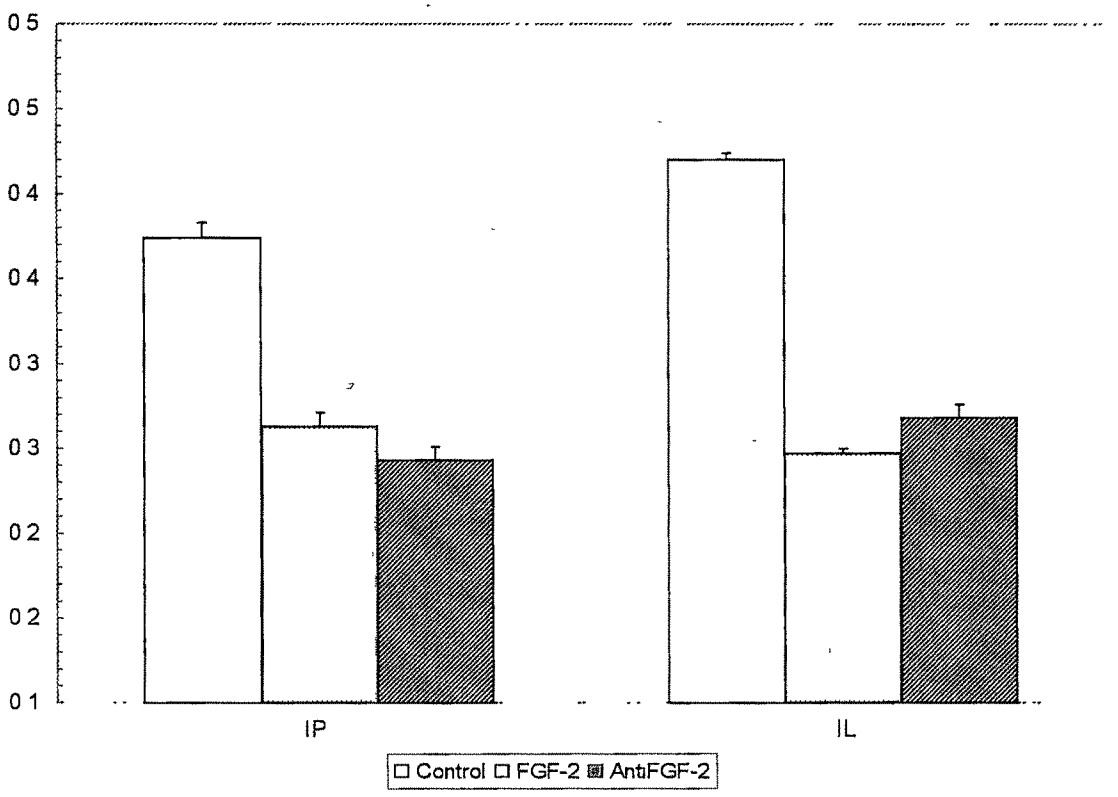

Figure. 2.11. Nucleic acid localization in regenerates of Control (Fig. 2.11a), FGF-2 (Fig.2.11b), and antiFGF-2 (Fig.2.11c) treated lizards at WE stage

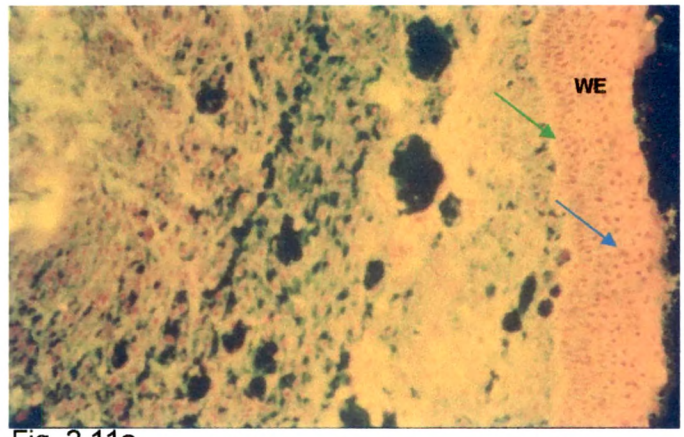

Fig. 2.11a

DNA localization → (Yellow fluorescence)

RNA localization → (Flame red fluorescence)

WE- wound epithelium

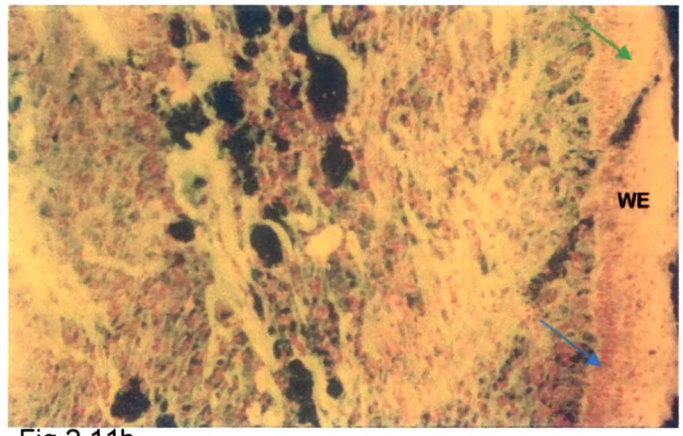

Fig.2.11b

DNA localization → (Higher Yellow fluorescence)

RNA localization → (Higher Flame red fluorescence)

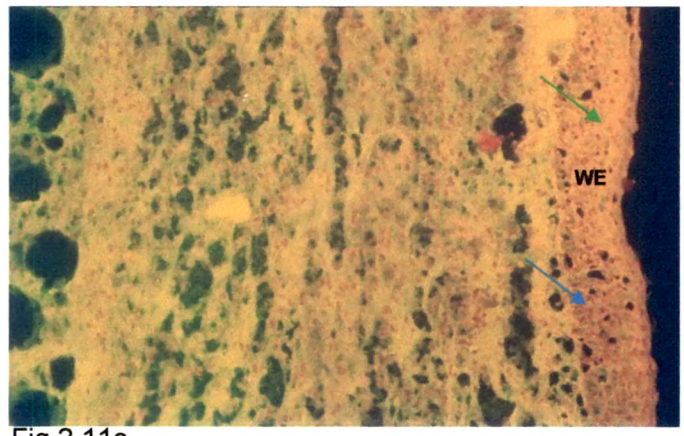

Fig.2.11c

DNA localization → (Less Yellow fluorescence)

RNA localization → (Less Flame red fluorescence)

Figure. 2.12. Nucleic acid localization in regenerates of Control (Fig. 2.12a), FGF-2 (Fig.2.12b), and antiFGF-2 (Fig.2.12c) treated lizards at BL stage

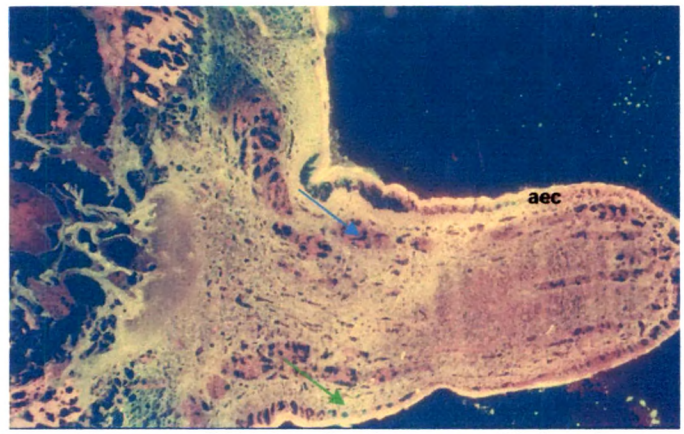

Fig.2.12a DNA localization → (Yellow fluorescence)
RNA localization → (Flame red fluorescence)
aec- apical epidermal cap

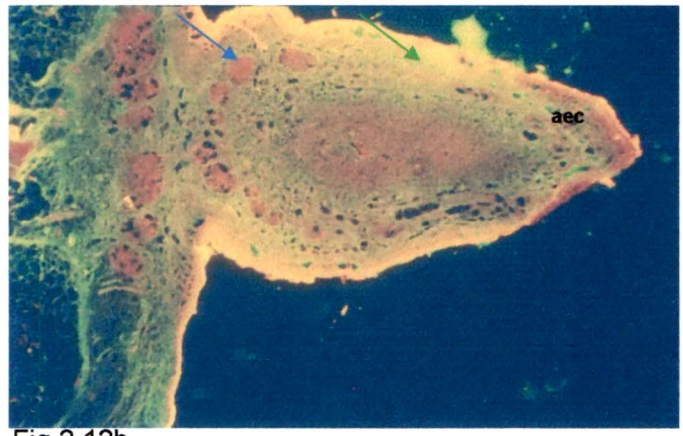

Fig.2.12b
DNA localization → (Higher Yellow fluorescence)
RNA localization → (Higher Flame red fluorescence)

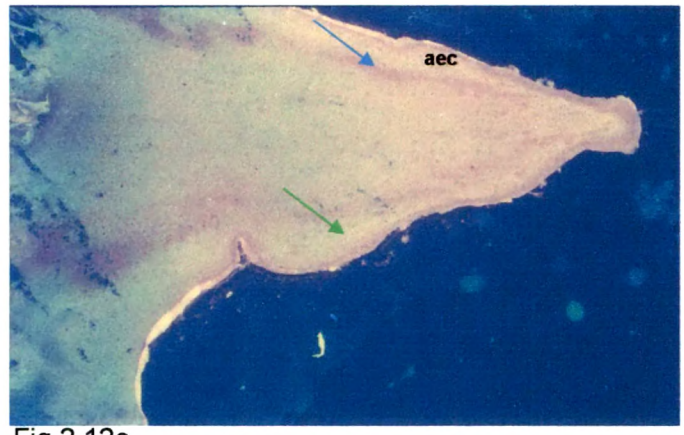

Fig.2.12c
DNA localization → (Less Yellow fluorescence)
RNA localization → (Less Flame red fluorescence)

Figure.2.13 Nucleic acid localization in regenerates of Control (Fig. 2.13a), FGF-2 (Fig.2.13b), and antiFGF-2 (Fig.2.13c) treated lizards at DF stage

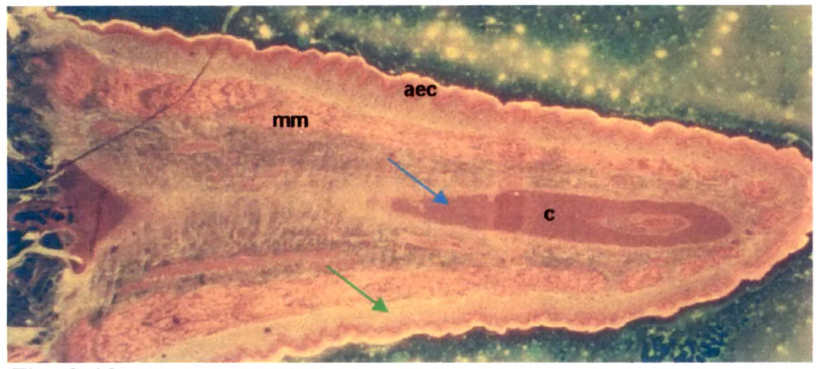

Fig. 2.13a

DNA localization → (Yellow fluorescence)

RNA localization → (Flame red fluorescence)

aec- apical epidermal cap

c- cartilage tube; mm- myomeres

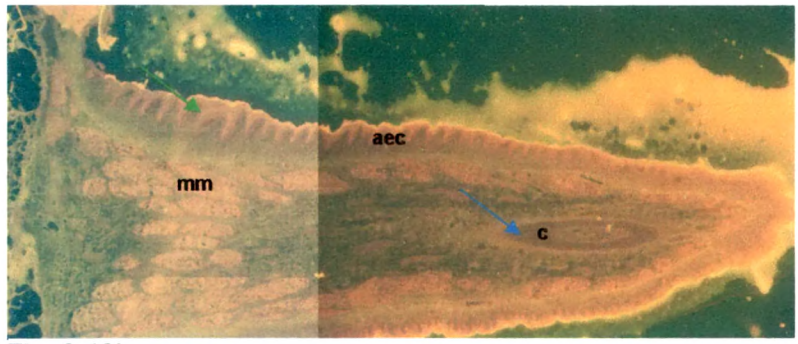

Fig. 2.13b

DNA localization → (Higher Yellow fluorescence)

RNA localization → (Less Flame red fluorescence)

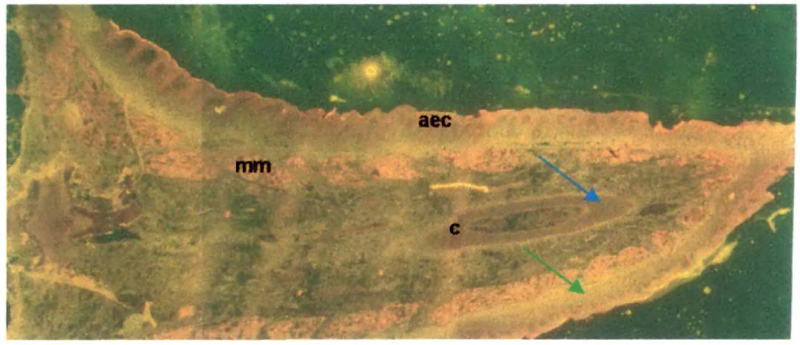

Fig. 2.13c

DNA localization → (Less Yellow fluorescence)

RNA localization → (Less Flame red fluorescence)