Expression of cell growth regulatory factor IGF-2 mRNA in sheep embryos

Submitted by

Avantika Mor

SRF (NFBSFARA AS2002)

and

PhD scholar (Biotechnology)

Major Guide

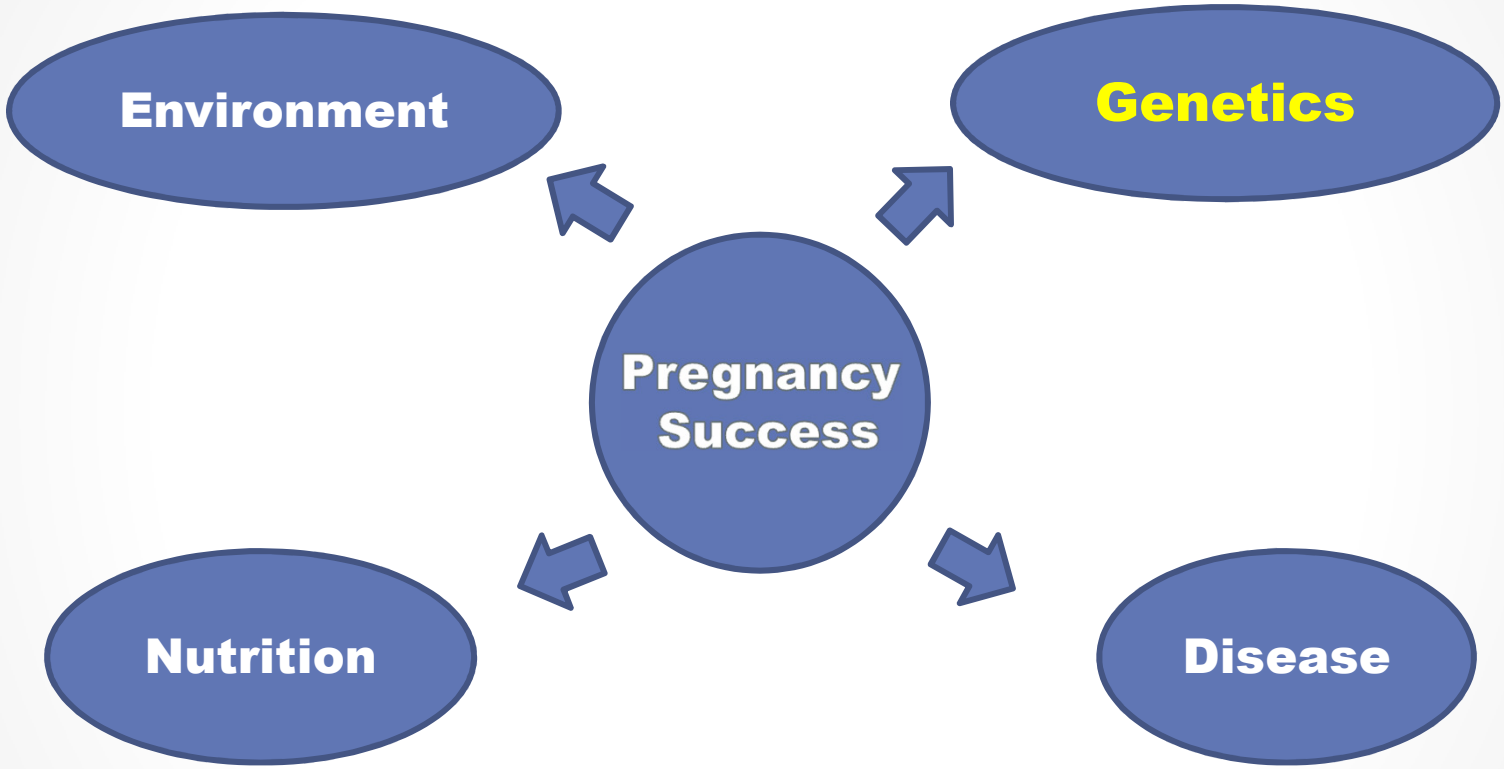
Dr. S. Mondal

Introduction

Early embryonic mortality is a major impediment hampering the reproductive efficiency of ruminants and accounts for a main component of post fertilization losses.

(Mondal and Varshney, 2007)

CHALLENGES TO EARLY PREGNANCY SUCCESS



- Genetics accounts for approximately one–third of the decrease in pregnancy rates.

  (Shook, 2006)

- **Profile of the genetic programme of normal development leading to a greater understanding of embryogenesis.**

- Aberration in expression of genes those are directly or indirectly regulate the development of the embryo, (like IGF-I, II and their receptor proteins IGF-1R, IGF-IIR and FGFs) affect the normal development of embryo.

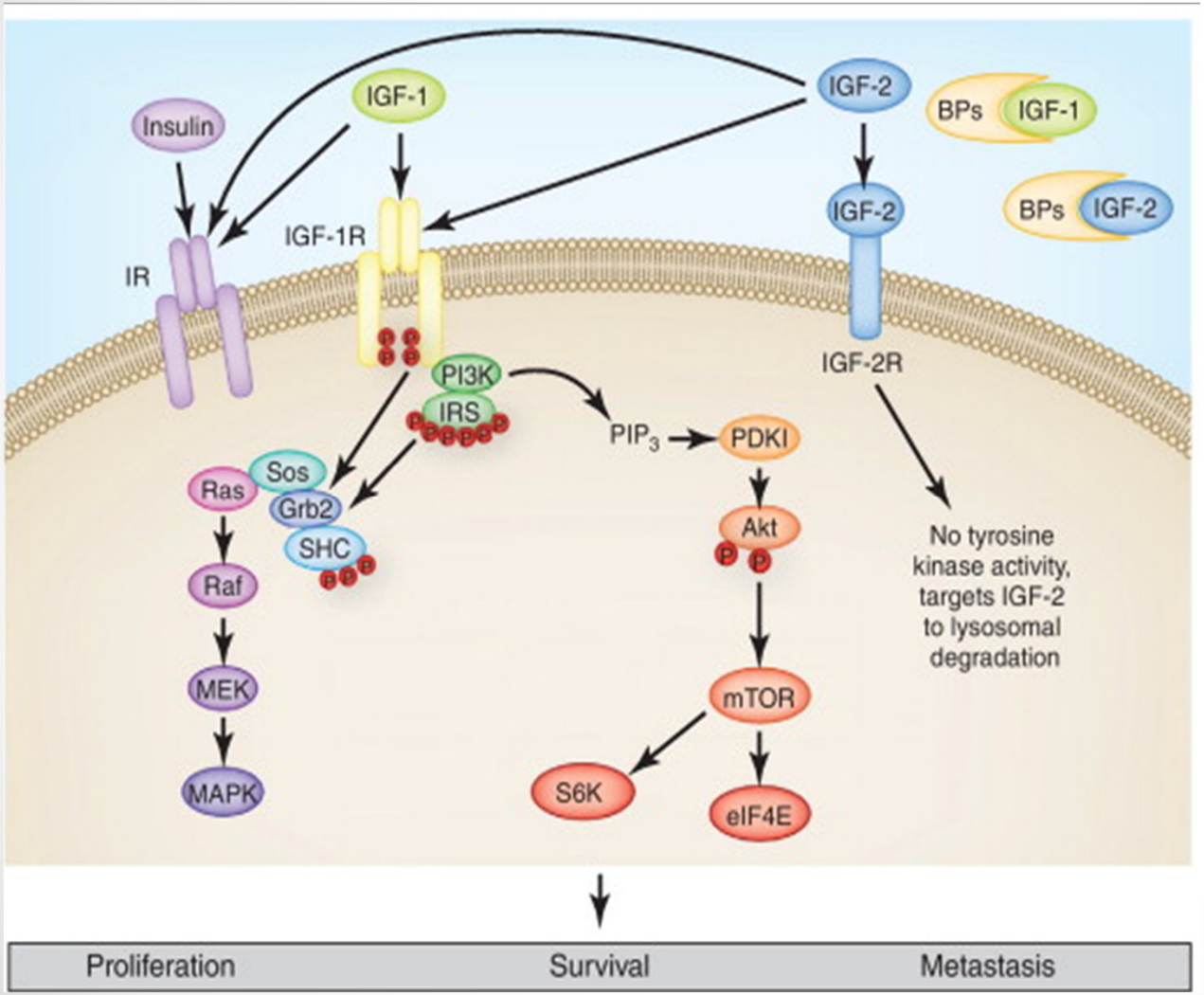
Objective

To study the expression profiling of insulin-like growth factor II (IGF-II) ligand and IGF-IIR receptor (IGF-IIR) in in vitro pre implantation sheep embryos.

IGFs (Insulin-like growth factors)

- IGFs are a group of structurally related polypeptides that regulate the growth of many types of mammalian cells.
- IGFI and IGF II are autocrine regulators of cell proliferation and paracrine growth and survival factor for mammalian embryo development.
- The growth-promoting activity of these polypeptides is mediated by binding to receptors on the cell membrane.

Overview of IGFs signalling



- The **Akt** Pathway, or PI3K-**Akt** Pathway is a signal transduction pathway that promotes survival and growth in response to extracellular signals.
- **Ras-Raf-MEK-ERK pathway** is a chain of proteins in the cell that communicates a signal from a receptor on the surface of the cell to the DNA in the nucleus of the cell

Growth factors play important role in modulating the embryonic growth and development.

Insulin-like growth factors (IGFs) and embryo development

- The IGFs complex system plays an essential role in normal human and animal development, including embryogenesis, pre- and postnatal growth and in the maintenance of tissue homeostasis.

  (Baker et al., 1993; Clemmons, 1997; Annunziata et al., 2011)

- The expression of IGF-I, II and their receptor proteins (IGF-1R and IGF-IIR) have been detected in ovary, oviducts, uterus and pre implantation embryos of bovine.

  (Daliri et al., 1999; Armstrong et al., 2000; Llewellyn et al., 2007; Sudo et al., 2007; Winger et al., 1997).

- IGF-II believed to be a major fetal growth factor in contrast to IGF-I which is a major growth factor in adults"

  (DeChiara et al., 1990)

- Expression of mRNA transcripts for the IGF family has been detected in embryos of a variety of species.

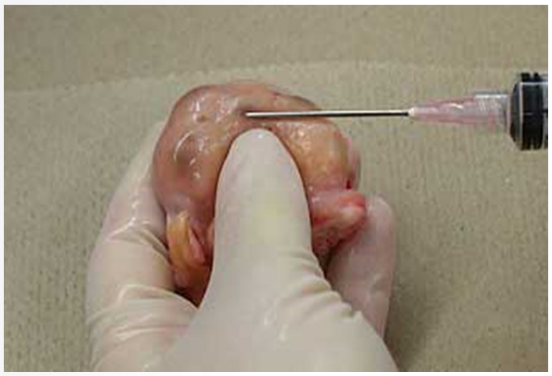
(watson et al.,1992; Yoshida et al. 1998)

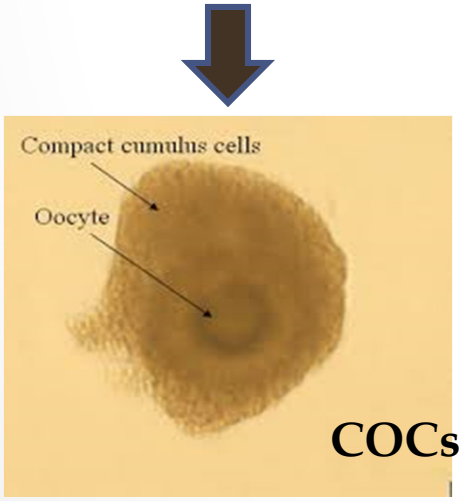
- IGF-I ,IGF-IR, IGF-II and IGF-IIR are expressed throughout pre implantation development up to the blastocyst stage.

(Schultz et al. 1992)

Methodology

Aspiration of oocyte (COCs) from slaughter house ovaries in aspiration media



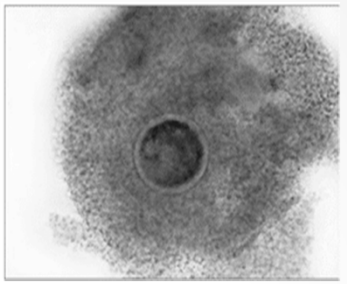


In vitro maturtion in maturation media in CO2 incubator

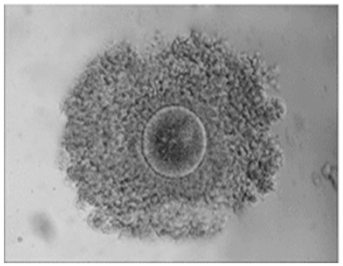


Grade 1: Compact cumulus mass having ≥5 layers of cumulus cells and with homogenous cytoplasm

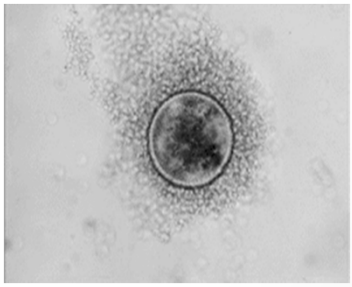
Grade 1: compact COCs with an unexpanded cumulus mass having ≥5 layers of cumulus cells and with homogenous cytoplasm



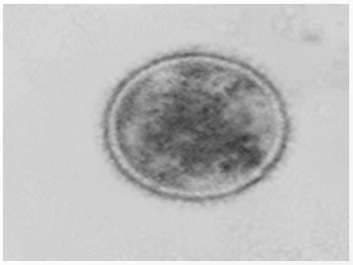
Grade 2: COCs with ≤4 layers of cumulus cells and with homogenous cytoplasm



Grade 3: oocytes with only 1-2 layers of irregular cumulus cells and with irregular cytoplasm.

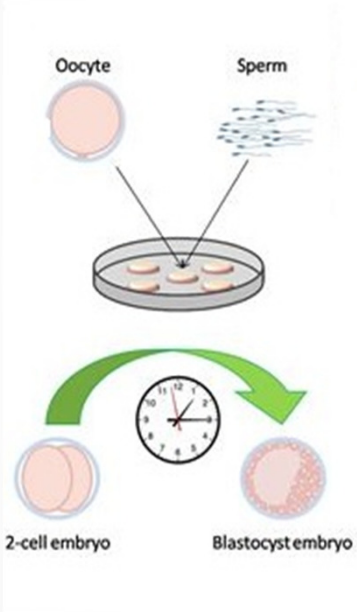


Grade 4: oocytes without cumulus cells and with irregular (shrunken) cytoplasm.



Methodology…

In vitro fertilization



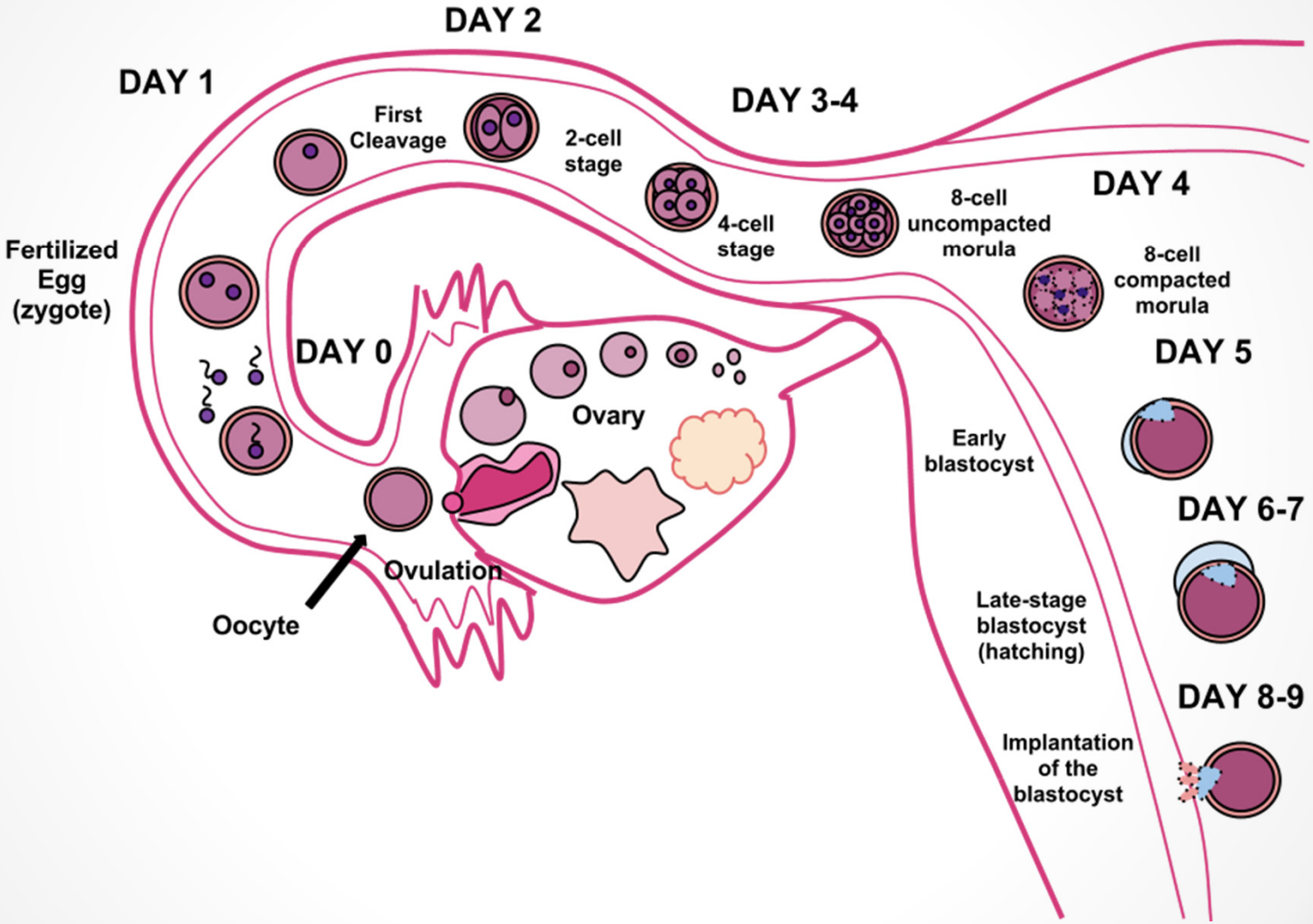
Collection of semen from ram by using electronic ejaculator

Capacitation of sperm by BO (Brackett and Oliphant) media

Matured oocytes were co-cultured with capacitated spermatozoa

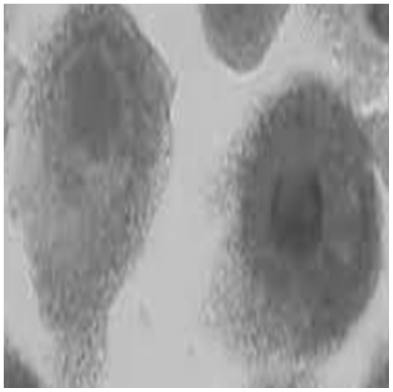
In vitro culture

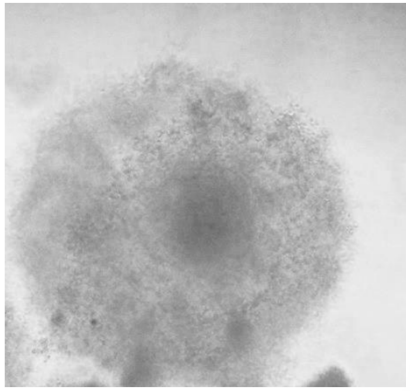
To confirm the fertilization and cleavage culture were checked approximately every 24 h for up to 6-7 days and observe the different stages of embryo development.


DAY 2
DAY 1
DAY 3-4
First Cleavage
2-cell stage
4-cell stage
DAY 4
8-cell uncompacted morula
8-cell compacted morula
Fertilized Egg (zygote)
DAY 0
Ovary
DAY 5
Early blastocyst
DAY 6-7
Ovulation
Oocyte
Late-stage blastocyst (hatching)
DAY 8-9
Implantation of the blastocyst

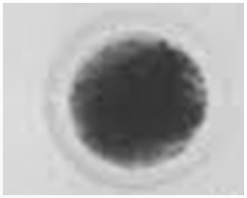
Methodology…

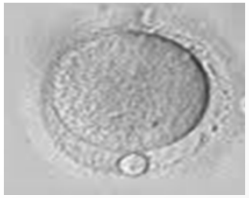
Collection of immature (n=100) and mature (n=100) oocyte in Trizol after removal of cumulus cells



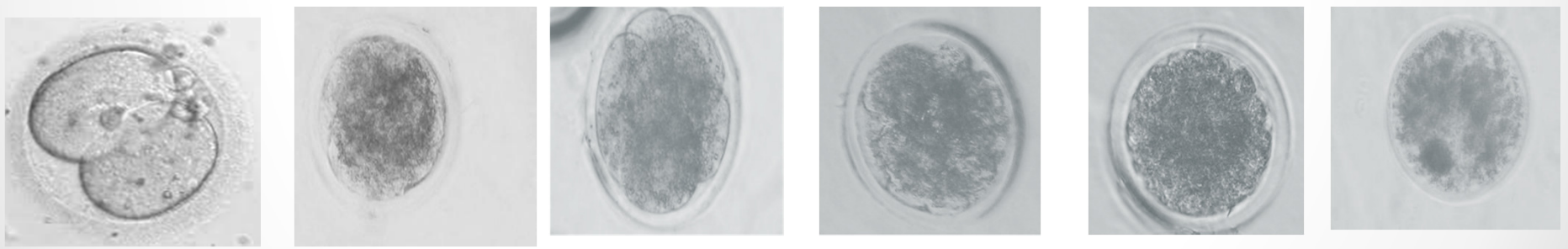


Denuded with 0.1% Hyaluronidase and votexing for 2-3min





Collection of different stages of embryo in Trizol, 2-cell (n=50), 4-cell (n=25), 8-cell (n=12) 16 cell (n=6) and for the morulae and blastocyst stages of embryos (n=5)



2 C 4 C 8 C 16 C Morula Blastocyst

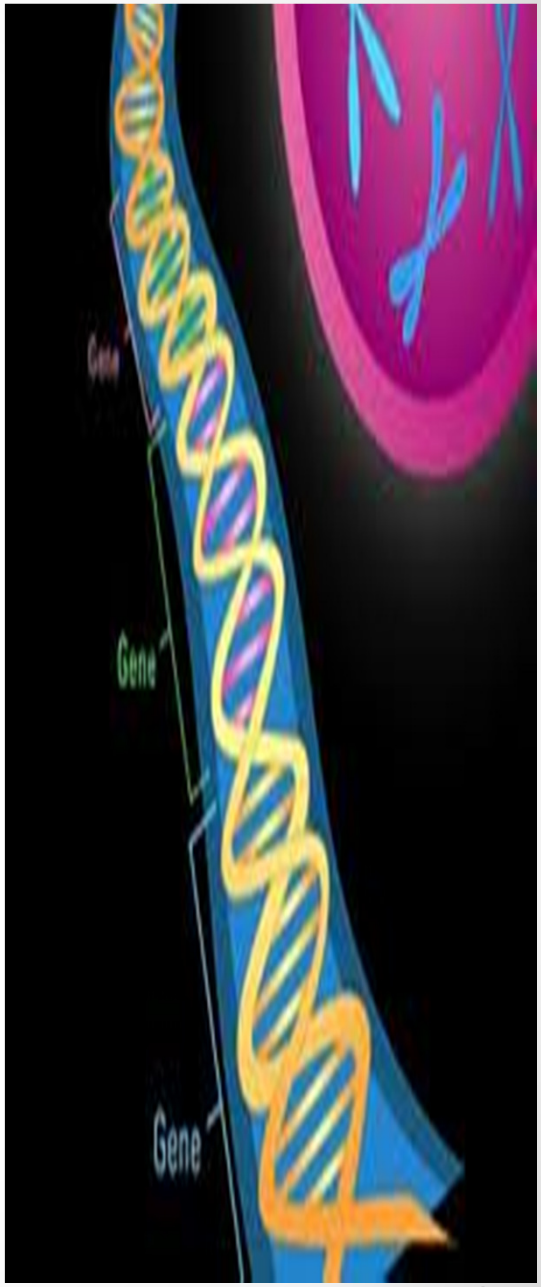
Developmental stages of sheep embryo

Methodology…

RNA was isolated from all stages of embryo by Trizol and kit (Qiagen minieasy RNA isolation kit) method

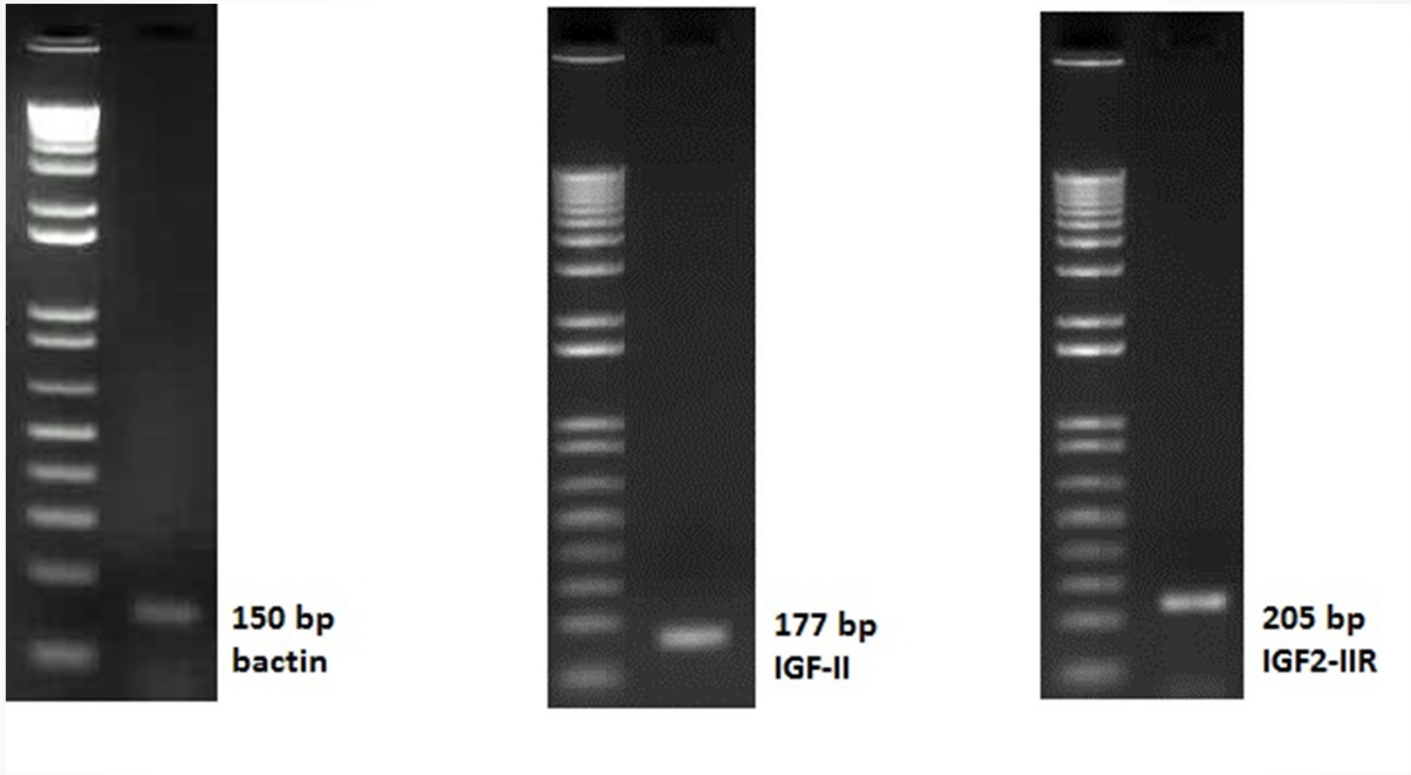
cDNA was prepared by Superscript III first strand synthesis kit (approximately 100ng RNA from each stage of embryo)

Expression studies were done by real time PCR using SYBR green dye



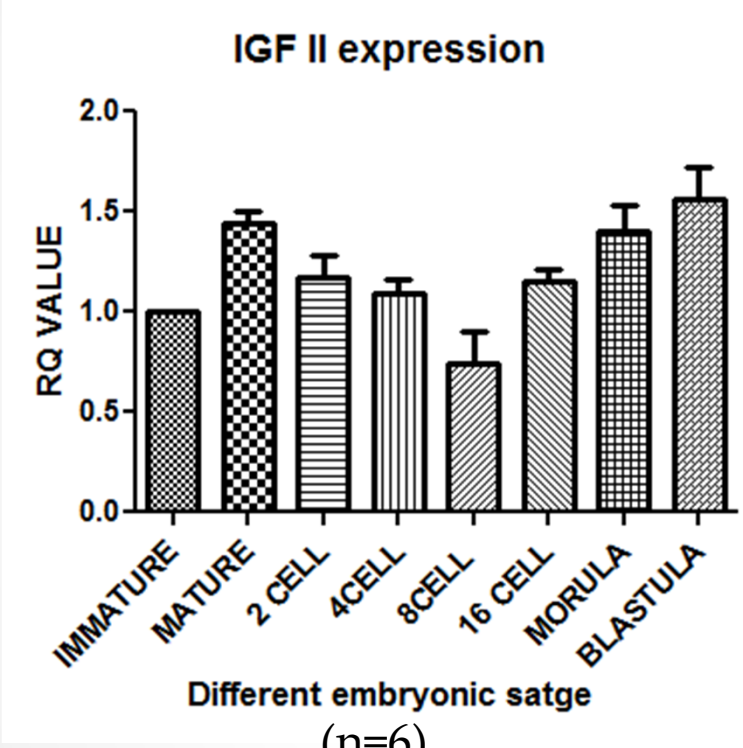
Primers used for real time PCR

Serial no	Gene	Primer (5′-3′)	Accesion no.	Amplicon size
1	B-actin FORWARD	AATTCCATGAAGTGACG	U39357.1	150bp
2	B-actin REVERSE	GATCTTGATCTTCATCGTGCTG		
3	IGF-II FORWARD	ACACCCTCCAGTTTGTCTGTG	NM_001009311.1	177bp
4	IGF-II REVERSE	GACTGCTTCCAGGTGTCAGATT		
5	IGF-IIR FORWARD	CACCTCACTGATCACGTTTCAC	AF327649.1	205bp
6	IGF-IIR REVERSE	CTTGAAGGTGTTCTTGGAGAGG		

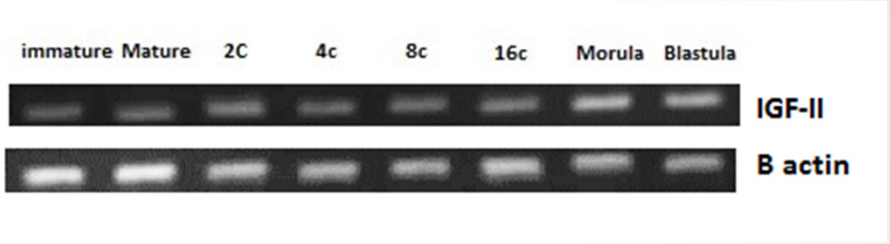


1% Agarose gel run for B-actin, IGF-II, IGF-IIR with 1kbp ladder

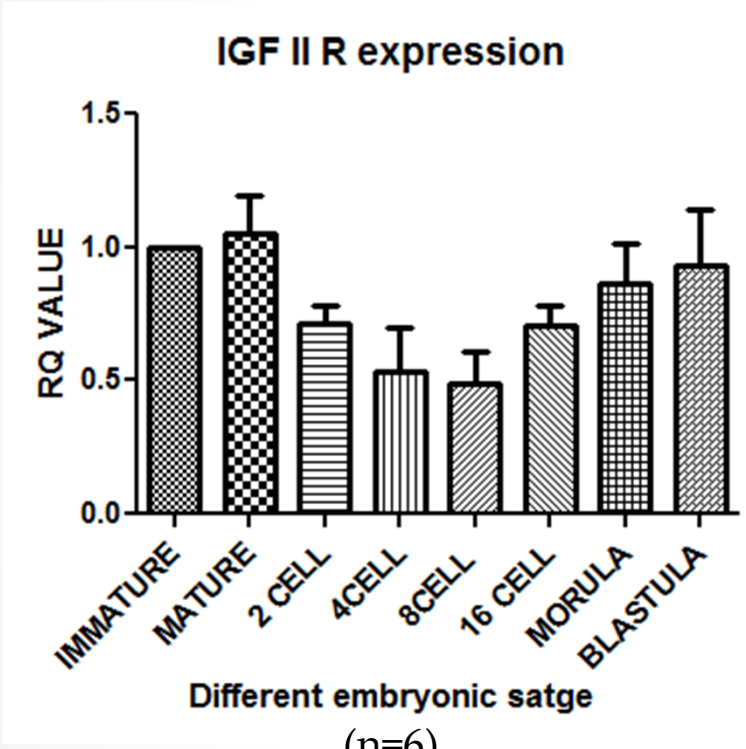
RESULT


IGF II expression
RQ VALUE
2.0
1.5
1.0
0.5
0.0
IMMATURE
MATURE
2 CELL
4CELL
8CELL
16 CELL
MORULA
BLASTULA
Different embryonic satge

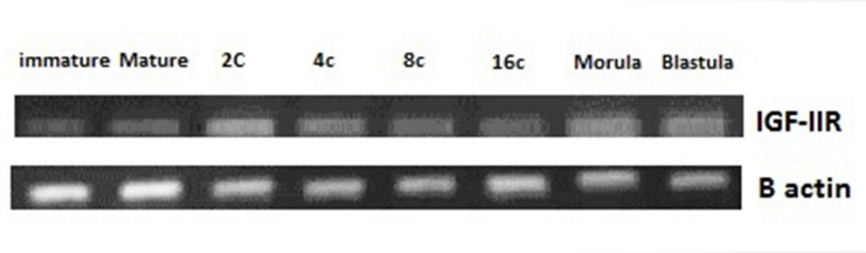
(n=6)

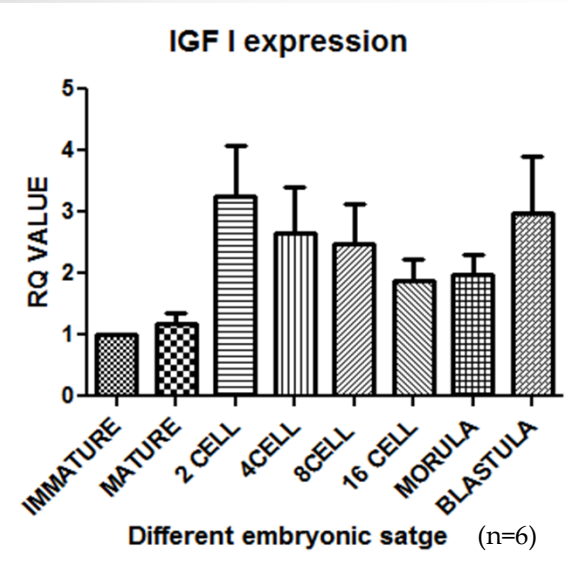

immature
Mature
2C
4c
8c
16c
Morula
Blastula
IGF-II
B actin

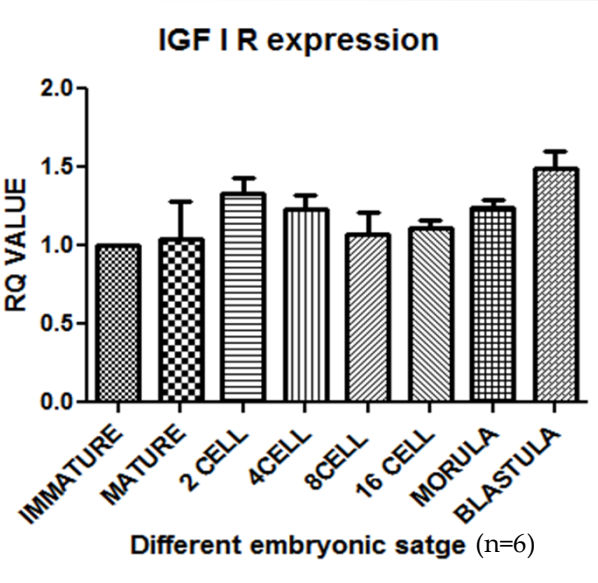
RESULT…

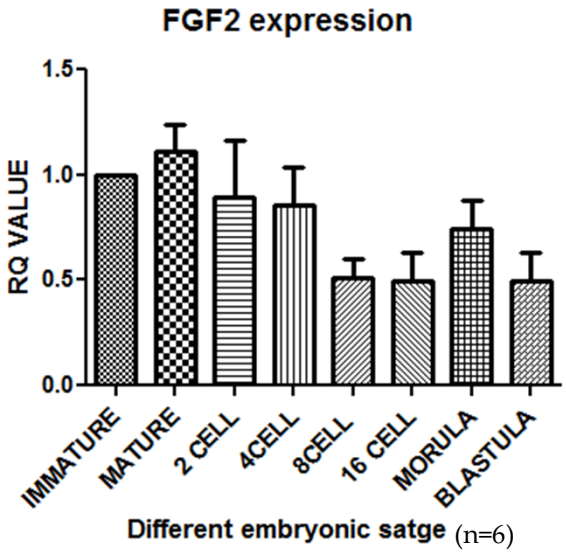

IGF II R expression
RQ VALUE
1.5
1.0
0.5
0.0
IMMATURE
MATURE
2 CELL
4CELL
8CELL
16 CELL
MORULA
BLASTULA
Different embryonic satge

(n=6)


immature Mature
2C
4c
8c
16c
Morula
Blastula
IGF-IIR
B actin


IGF I expression
RQ VALUE
0
1
2
3
4
5
IMMATURE
MATURE
2 CELL
4CELL
8CELL
16 CELL
MORULA
BLASTULA
Different embryonic satge (n=6)


IGF I R expression
RQ VALUE
0.0
0.5
1.0
1.5
2.0
IMMATURE
MATURE
2 CELL
4CELL
8CELL
16 CELL
MORULA
BLASTULA
Different embryonic satge (n=6)


FGF2 expression
RQ VALUE
0.0
0.5
1.0
1.5
IMMATURE
MATURE
2 CELL
4CELL
8CELL
16 CELL
MORULA
BLASTULA
Different embryonic satge (n=6)

Conclusion

IGF-II and IGF-IIR expressions showed that gene was activated at all stages in IVF embryos and expression level was decreased from the 2-cell stage to up to 8-cell stage and after that continuous increase was observed up to blastula stage.

Acknowledgement

- **NFBSFARA**(National Fund for Basic, Strategic and Frontier Application Research in Agriculture) **National Agriculture science Fund**

- Guide Dr. S. Mondal (Principal Scientist) and Dr. I.J. Reddy, Dr. P S P Gupta, Dr. S. Nandi

- Director, NIANP and Director, Jain university


Thank
You