Neutrophilic Toll Like Receptor 4, Fas Gene Expression and Level of Some Plasma Parameters: Causative Factors for Repeat Breeding

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INTRODUCTION

3 TIMES AI

NO CONCEPTION

REPEAT BREEDER

{COW THAT HAS NOT CONCEIVED AFTER THREE OR MORE SERVICES}

- **IGF-1**
  - REPRODUCTION
  - IMMUNITY
  - INFLAMMATION
- **PLASMA LACTOFERRIN** / **PLASMA HAPTOGLOBIN** → IMMUNE STATUS OF THE ANIMAL
- **TLR -4** → ACTIVATE INNATE IMMUNITY → PATHOGEN RECOGNITION
- **Fas** Genes → DEATH RECEPTOR → NEUTROPHIL APOPTOSIS

OBJECTIVE

The present study was conducted in order to investigate whether the **TLR-4** and **Fas gene expression** in neutrophils and plasma level of **IGF-1, Lactoferrin (LF)** and **Haptoglobin (Hp)** varied between **repeat breeding (RB)** and **regular breeding (R_gB)** cross bred cattle.

MATERIALS AND METHODS

Selection of animals

Reared under farm and field conditions at Karnal, Haryana, India

- Recently calved Karan Fries cattle
- Free from clinical infections
- 2nd -3rd parity; 400-460 kg body wt

Post partum cows monitored up to three consecutive services

- Conceived → Regular breeders (R_gB) (n=6)
- Not Conceived → Repeat breeders (RB) (n=14)

Study Period = 4th -21st week postpartum
Collection of blood at weekly intervals (stopped when animal got pregnant)

Separation of blood plasma

Estimation of plasma concentrations of:
- **IGF-1**
- **Lactoferrin (LF)**
- **Haptoglobin (Hp)**

Concentration of cell suspension **(neutrophils)** used: **5 X 10^6 live cells/ml** in culture media

Viability determination by **Trypan blue exclusion method**

Separation of neutrophils

Culture of neutrophils for 2h

- 200µl of Cell suspension per well in triplicate in 96 well plates
- **MEDIA:** RPMI 1640 containing 10% FCS, 650 µg/ml Zymosan, 250 µg/ml NBT (5% CO_2, 37˚C)

R_gB | RB | RB

In vitro **IGF- 1** supplementation **@ 100ng/ml**

TLR 4 AND Fas GENE RELATIVE EXPRESSION

[**GAPDH** was kept as house keeping gene]

DETAILS OF PRIMERS

GENES	SEQUENCE (5' → 3')	SIZE (bp)
TLR 4	F GGCATCATCTTCATCGTCCT	178
	R CTGGACTCTGGGGTTTACCA	
Fas	F GAAGAGGAGGGACCACA	188
	R TGGGGTGACCTATTGCT	
GAPDH	F GGGTCATCATCTCTGCACCT	176
	R GGTCATAAGTCCCTCCACGA	

RESULTS

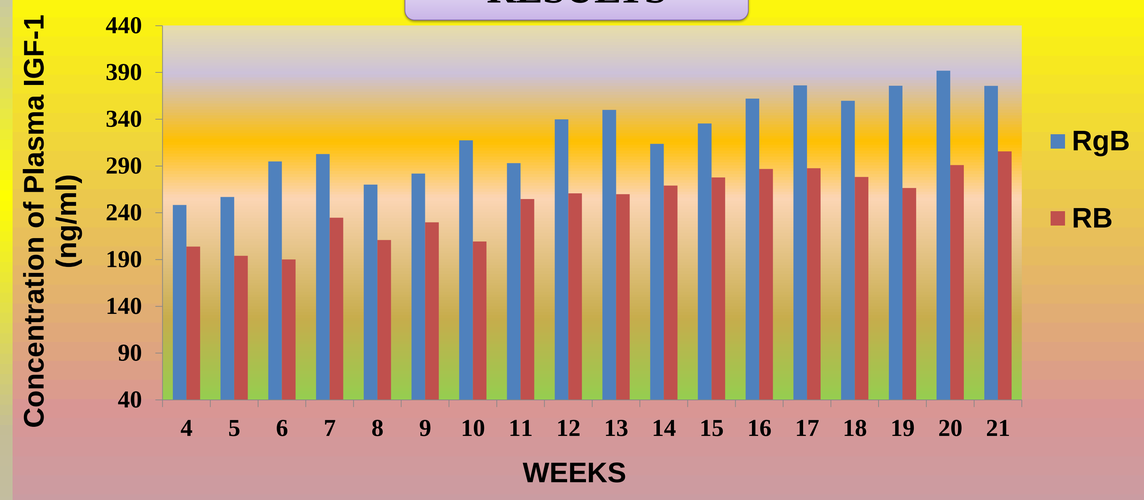

FIG. 1: Concentration of Plasma IGF- 1 in RgB and RB groups

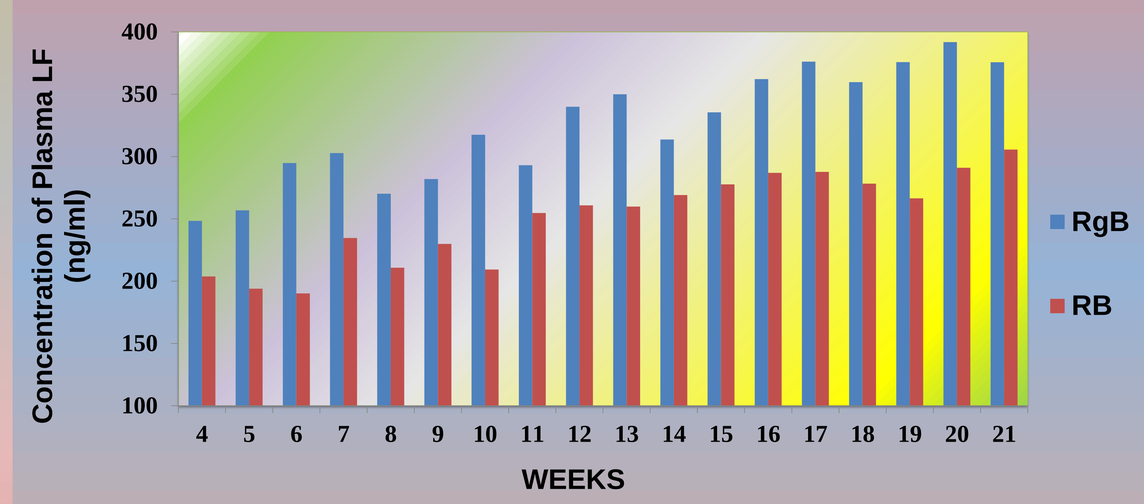

FIG. 2: Concentration of Plasma Lactoferrin in RgB and RB groups

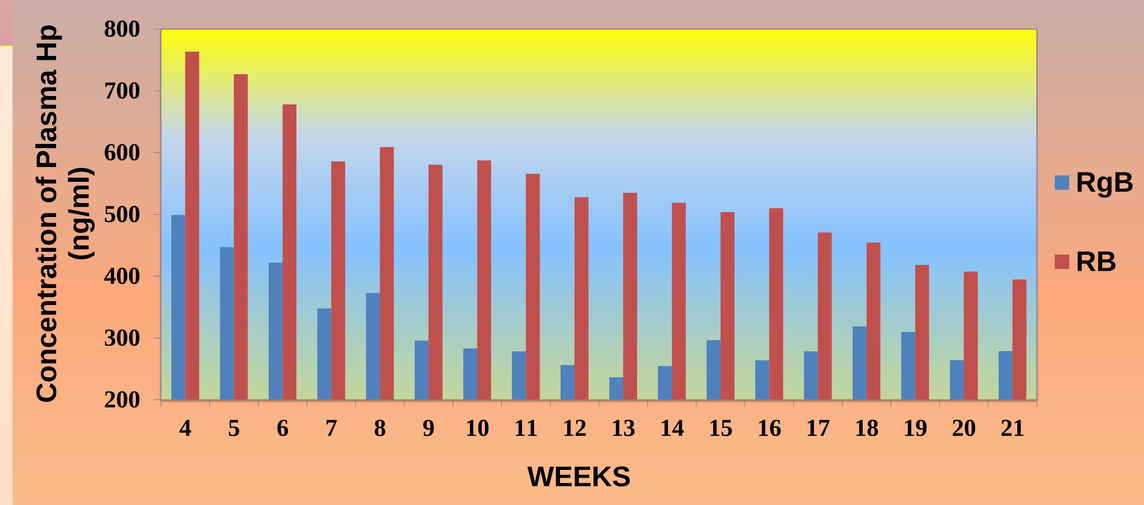

FIG. 3: Concentration of Plasma Haptoglobin in RgB and RB groups

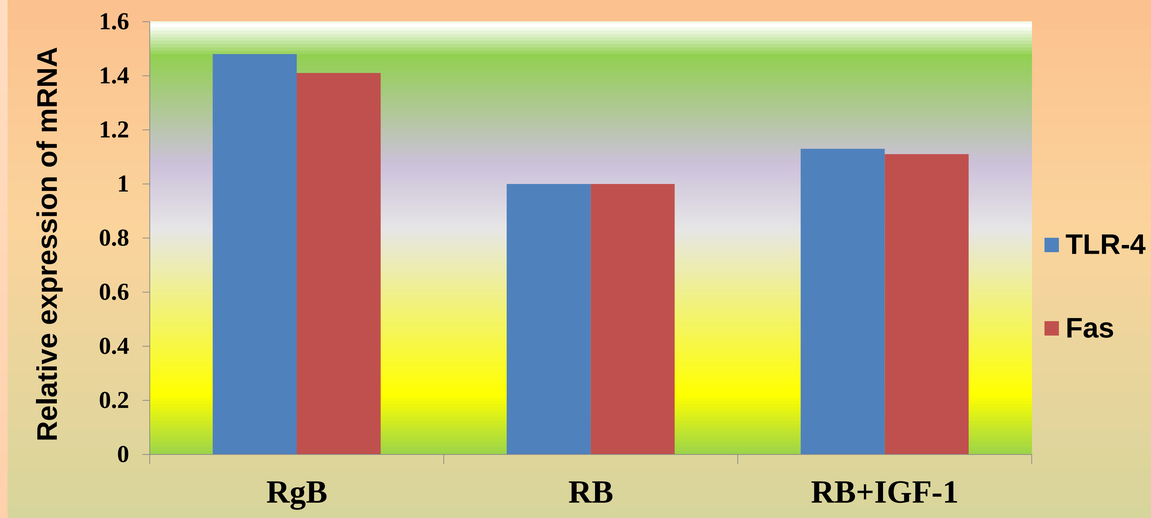

FIG. 4: Relative expression of TLR-4 and Fas mRNA in neutrophils of RgB, RB and RB (*In vitro* IGF-1 supplemented) groups

CONCLUSION

From the present study, it was concluded that the **immune status of R_gB group animals was better when compared with RB group animals** and **poor immune status may be one of the causative factor for repeat breeding problem.**