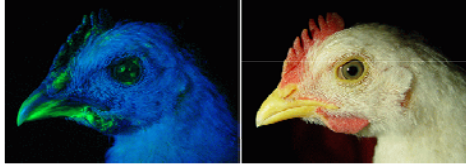




## Transgenic Chicken as Bioreactor for Producing Pharmaceutical Proteins

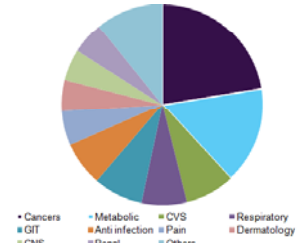


*Prof. Mukesh K Gupta*

National Institute of Technology Rourkela, Odisha

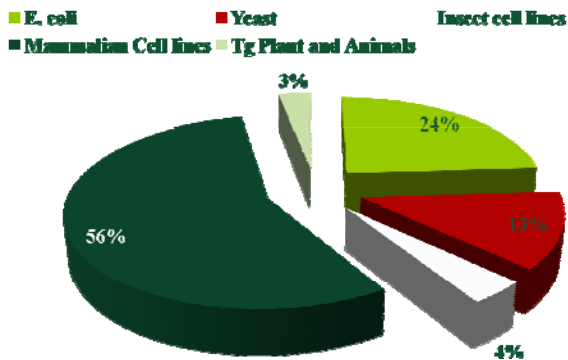
## Global Market of Pharmaceutical Proteins

- Global Market : **US\$ 65 Billion**
- CAGR : **9.4%**
- Dominating drugs : **46**
- Peptide drugs under clinical trials: **807**



Source: GVR, USA

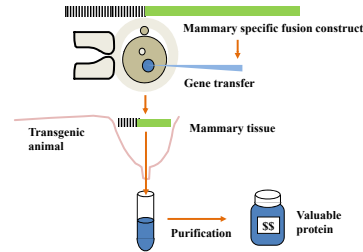
## Pharmaceutical Protein Production



Trend in new drug filings for FDA approval

Source: BioPharma Int.

## Animals as Bioreactor



BOLAM (1997, Lactoferrin)

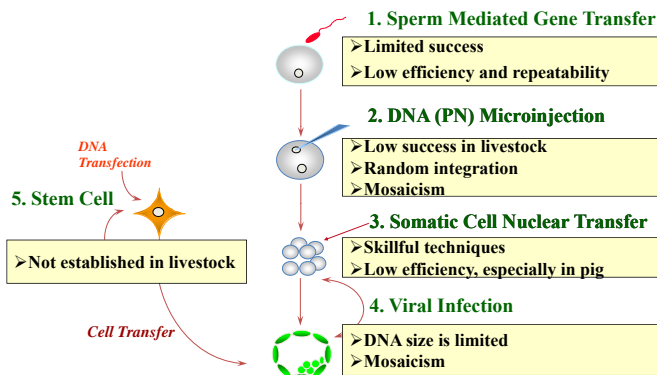


MEDDY (1998, G-CSF)



Saeromi (1999, EPO)

## Strategies for Producing Transgenics?

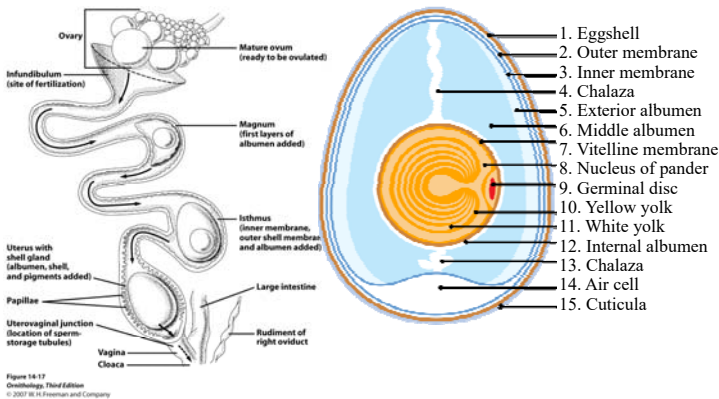


## Chicken as Bioreactor



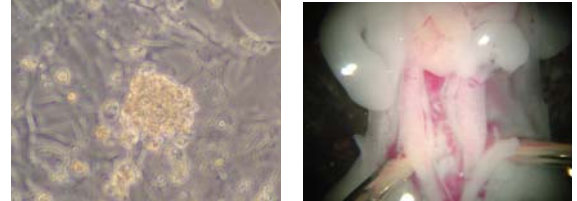
- **Productivity**
  - Short generation
  - Many offspring
- **High protein in Egg**
- **Ease of purification**
  - 8 kinds proteins

## Problem with Chicken Transgenesis

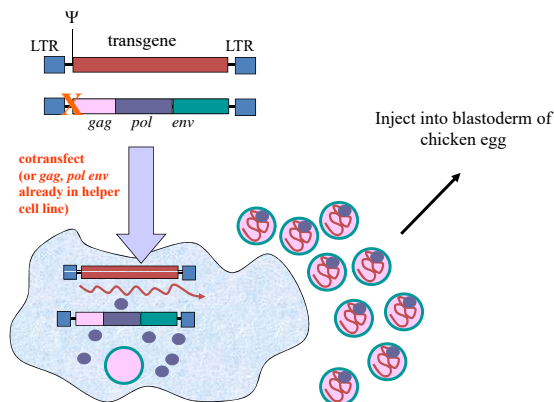


## Strategies of Chicken Transgenesis

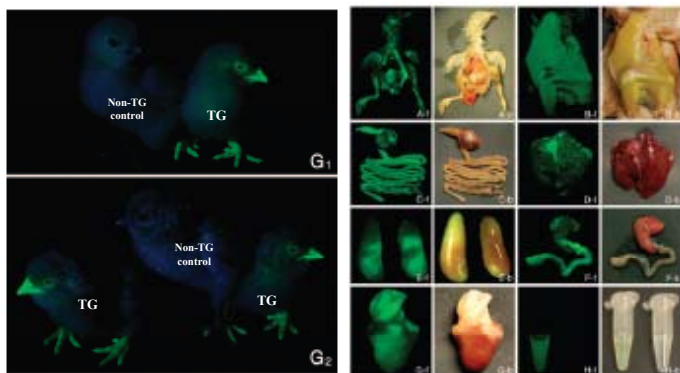
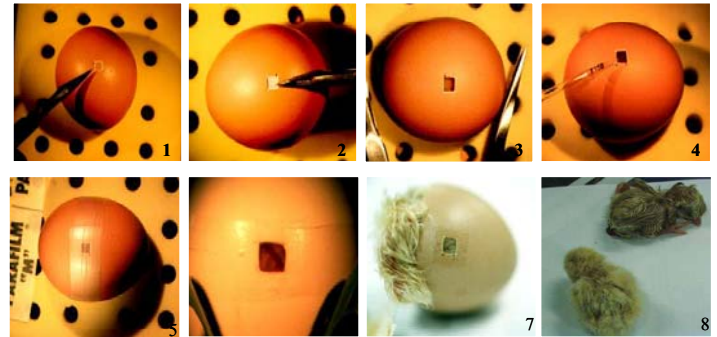
- **Blastoderm cell** *Iches et al. (1993)*
- **Primordial Germ cell** *Wentworth et al. (1993)*
- **Retroviral vector** *Savva et al. (1991)*
- **Lentiviral vector** *Sang et al. (2004)*



## Chicken Transgenesis – Retroviral Vector



## Cell injection by Windowed egg method



Expression of eGFP gene in G1 and G2 Tg chickens

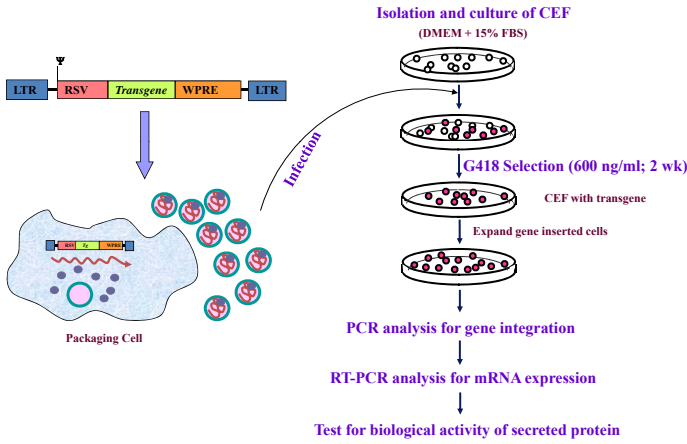
Expression of eGFP gene in various organs of G1 and G2 Tg chickens

(FASEB J., 2006)

## huPA and hPTH Transgenic Chicken

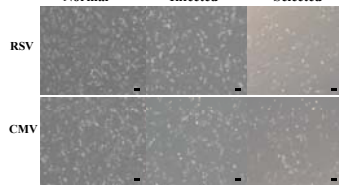


# huPA and hPTH Transgenic Chicken

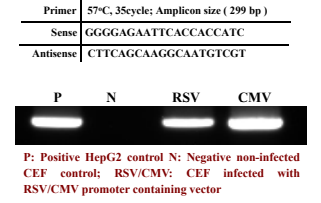


# huPA Transgenic Chicken

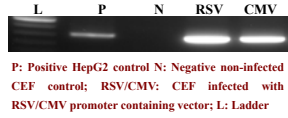
## Infection of CEFs with recombinant Retrovirus (huPA)



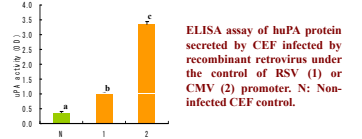
## Confirmation of gene integration in CEF (huPA)



## Expression of huPA transcript in infected CEF



## Biological activity of huPA secreted by CEF



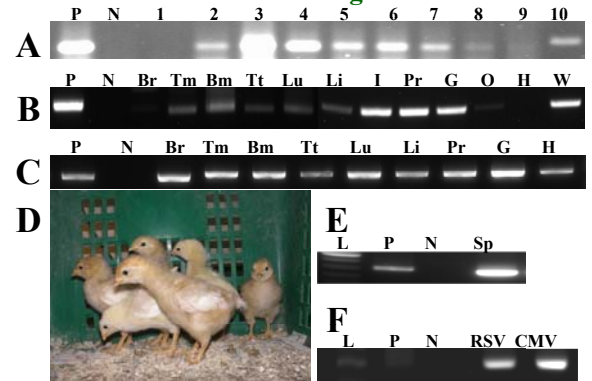
# huPA Transgenic Chicken

## Survival and hatching rates of manipulated chicken eggs

Groups	No. of embryos	No. (%) of embryos surviving	
		18 days	Hatched
Control	1087	952(87.6)	884 (81.3)
Windowed	618	448 (72.5)	371 (60.0)
Inj. DMEM	603	397 (65.8)	236 (39.1)
Inj. LN-RSV-Uro-W	382	141 (37.3)	30 (8.2)
Inj. LN-CMV-Uro-W	191	57 (30.2)	8 (4.2)

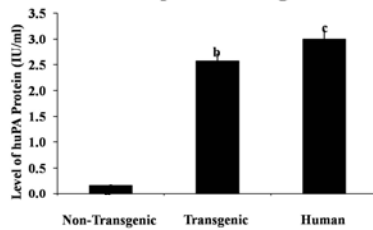
✓RSV promoter was superior to CMV both in terms of expression level and viable offspring

## Detection of huPA gene (A and B) and transcripts (C) in G0 huPA transgenics

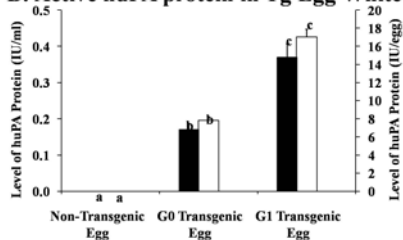


A: Genomic DNA were isolated from wing and toe of chicks (1-10). B: Genomic DNA were isolated from different organs. C: Total RNA were isolated from different organs of transgenic chicks. D: Young chicks; E: Total RNA was isolated from sperm of adult rooster. F: Expression of huPA transcript under the control of RSV or CMV promoter. Br: Brain, Tm: Thigh muscle, Bm: Breast muscle, Tt: Testis, Lu: Lung, Li: Liver, I: Intestine, Pr: Proventriculus, G: Gizzard, O: Oviduct, H: Heart, W: Wing tip, Sp: Sperm. For positive (lane P) and negative (Lane N) controls, known quantities of genomic DNA (A and B) or total mRNA (C) isolated from virus packaging cell and a non-transgenic chicken, respectively were used.

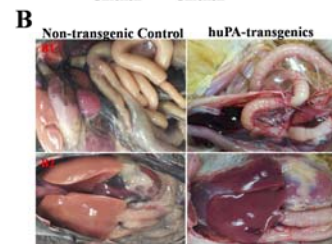
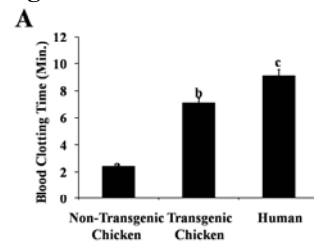
## A: Active huPA protein in Tg blood



## B: Active huPA protein in Tg Egg White



## Blood clotting disorder in huPA transgenic chicken



## Reproductive Performance of huPA Transgenics



### Mean survival and hatching rate of G1 eggs from huPA transgenic chicken

Groups	No. of eggs	No. (%) of survived embryos at			No. (%) of transgenic chicks
		Day 9	Day 18	Hatching	
Non-transgenic Chicken	288	264 (91.7) <sup>a</sup>	241 (83.7) <sup>a</sup>	212 (73.6) <sup>a</sup>	-
Transgenic Chicken	341	180 (52.8) <sup>b</sup>	153 (44.9) <sup>b</sup>	92 (27.0) <sup>b</sup>	36 (39.1)

Values in parenthesis indicate the number of eggs.  
Values with different superscripts (a, b, c) within a column differ significantly (p<0.05).

## Semen parameters (Mean ± SEM) in transgenic chicken expressing the huPA

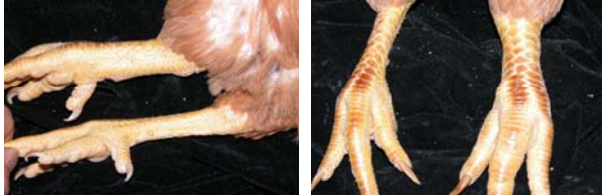
Groups	Volume of Ejaculate (µl)	Sperm Conc. (x10 <sup>9</sup> /ml)	Sperm Viability (%)
Non-transgenic Chicken	480.0 <sup>a</sup> ± 20.0	3.4 <sup>a</sup> ± 0.2	80.0 <sup>a</sup> ± 0.0
Transgenic Chicken	200.0 <sup>b</sup> ± 13.8	0.8 <sup>b</sup> ± 0.0	54.0 <sup>b</sup> ± 4.0

Experiments were replicated five times.  
Values with different superscripts (a, b) within a column differ significantly (p<0.05).

### Normal transgenic (huPA) chicken



### Abnormality in transgenic (hPTH) chicken

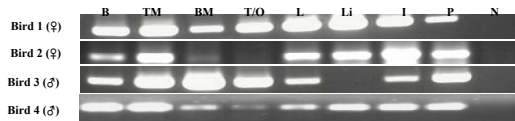


## hPTH Transgenic Chicken

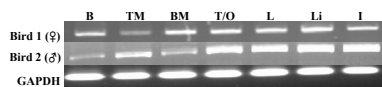
### Survival and hatching rates of manipulated chicken eggs

Groups	No. of eggs	Survival rate on		Hatching rate
		Day 9	Day 18	
Control	870	93.1 <sup>a</sup> ±1.5 (803)	88.5 <sup>a</sup> ±1.9 (757)	83.1 <sup>a</sup> ±2.9 (698)
Windowed	410	90.3 <sup>a</sup> ±1.0 (369)	81.4 <sup>a</sup> ±1.1 (332)	70.1 <sup>a</sup> ±1.6 (287)
DMEM injected	480	87.5 <sup>a</sup> ±2.1 (419)	71.7 <sup>a</sup> ±2.8 (345)	42.7 <sup>b</sup> ±2.0 (204)
hPTH injected	473	66.8 <sup>b</sup> ±1.2 (473)	22.6 <sup>b</sup> ±1.5 (110)	8.3 <sup>c</sup> ±2.0 (42)

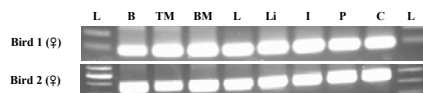
Values in parenthesis indicate the number of eggs.  
Values with different superscripts (a, b, c) within a column differ significantly (P<0.05).



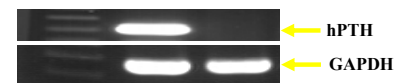
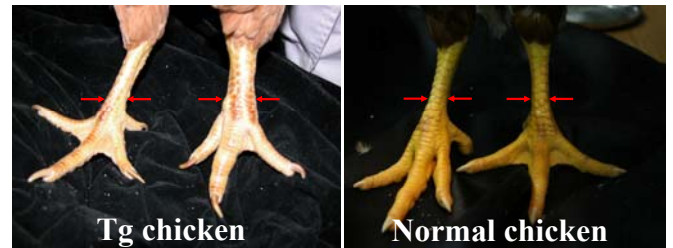
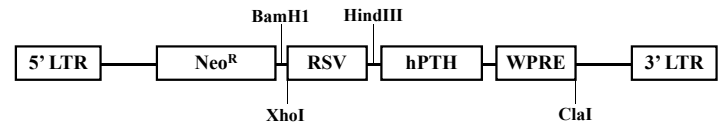
Detection of human parathormone gene in G0 transgenic chickens.



Detection of human parathormone transcripts in G0 transgenic chickens.



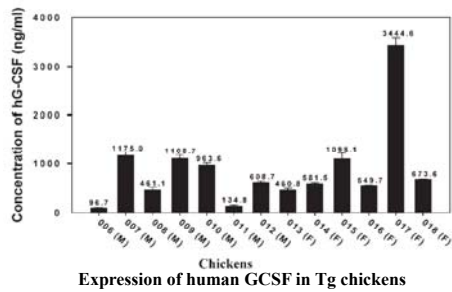
Detection of human parathormone gene in G1 transgenic chickens.



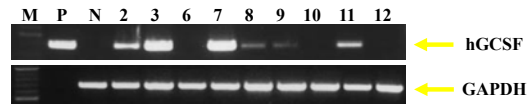
Expression of human parathormone on tg chicken



## hGCSF Transgenic Chicken



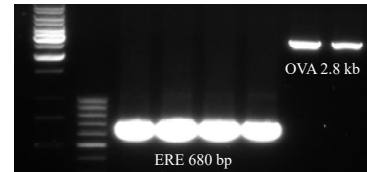
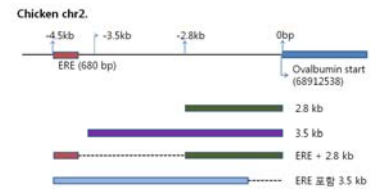
Expression of human GCSF in Tg chickens



Detection of human GCSF in Tg chicken (Kwon et al., MRD., 2008)

## Egg-specific Expression of Transgene (hEGF)

- Ovalbumin constitutes ~54% of the protein in the egg-white



- 2.8 OVA
- 3.5 OVA
- ERE-OVA : size 3.5 kb

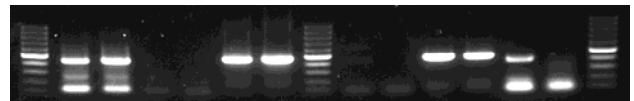
※ Four kinds viral vector constructed with different promoter sequence

## hEGF Transgenic Chicken

### Survival and hatching rates of manipulated chicken eggs

Groups	No. of embryos	No. (%) of survived embryos	
		18 days	Hatched
Control	65	59 (90.8)	56 (86.2)
Windowed	144	80 (55.6)	66 (45.8)
2.8 kb size	161	54 (33.5)	20 (12.4)
3.5 kb size ERE +	161	59 (36.6)	22 (13.7)
3.5 kb size ERE -	161	47 (29.2)	24 (14.9)
ERE + 2.8 kb size	161	52 (32.3)	25 (15.5)

- Integration of *hEGF* in G<sub>0</sub> chicks



G<sub>0</sub> chicks

## Conclusion

- ✓ Transgenic chicken are excellent bioreactor for
  - Producing human proteins of medicinal value
  - Study developmental effect of human proteins
- ✓ Specific expression in egg may pave way for large scale commercial production
- ✓ Value Addition to Poultry Production

Thank You