The role of growth factors in regulating cellular events during ovarian follicular development

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SESSION #54 EAAP 2015

Outline:

General/Background on:

IGF, TGFB, Hh, FGFs Systems

Data on:

IGFs; GDFs, BMPs; Hhs; FGFs &

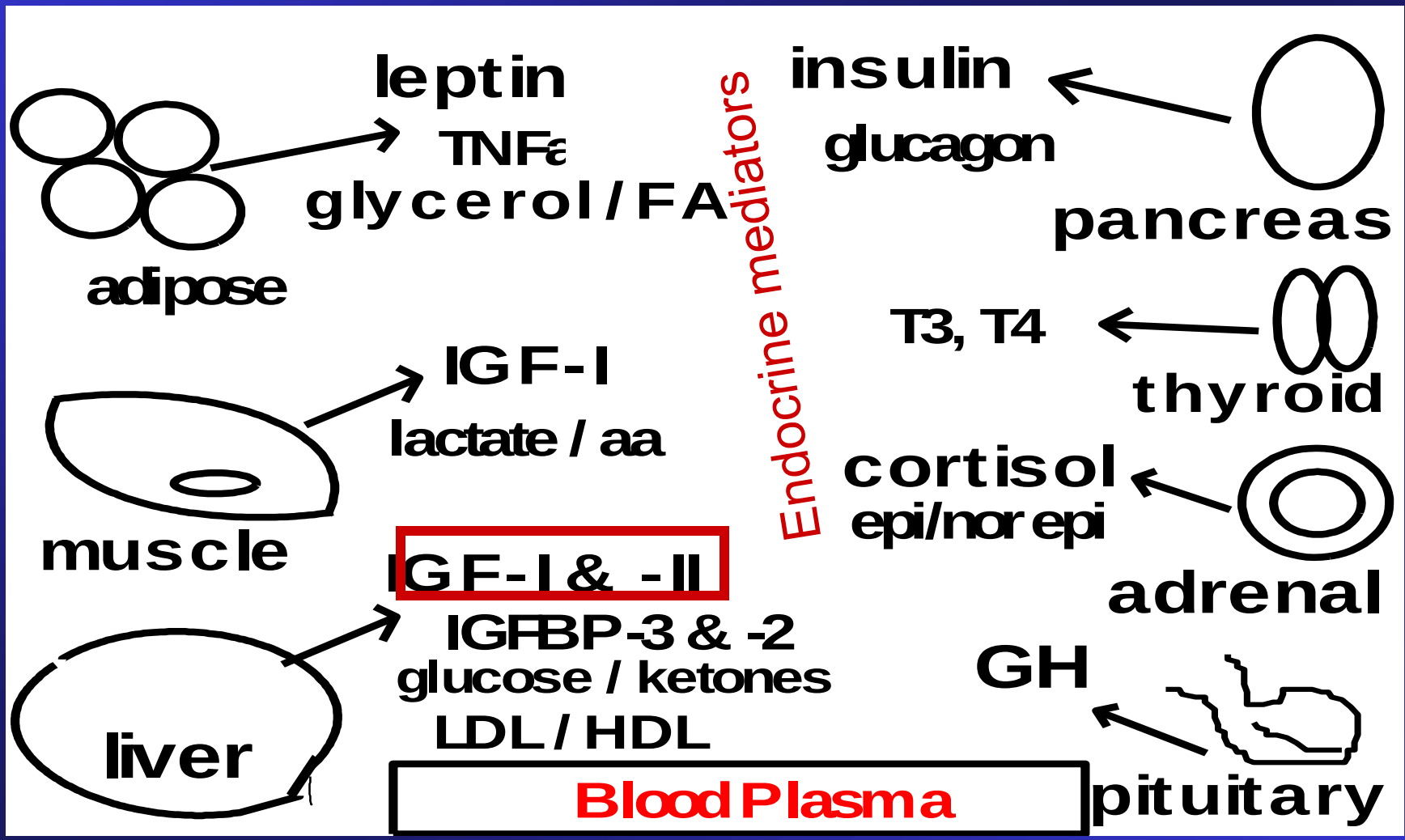
Ovarian Follicle Development

Future Directions

Background

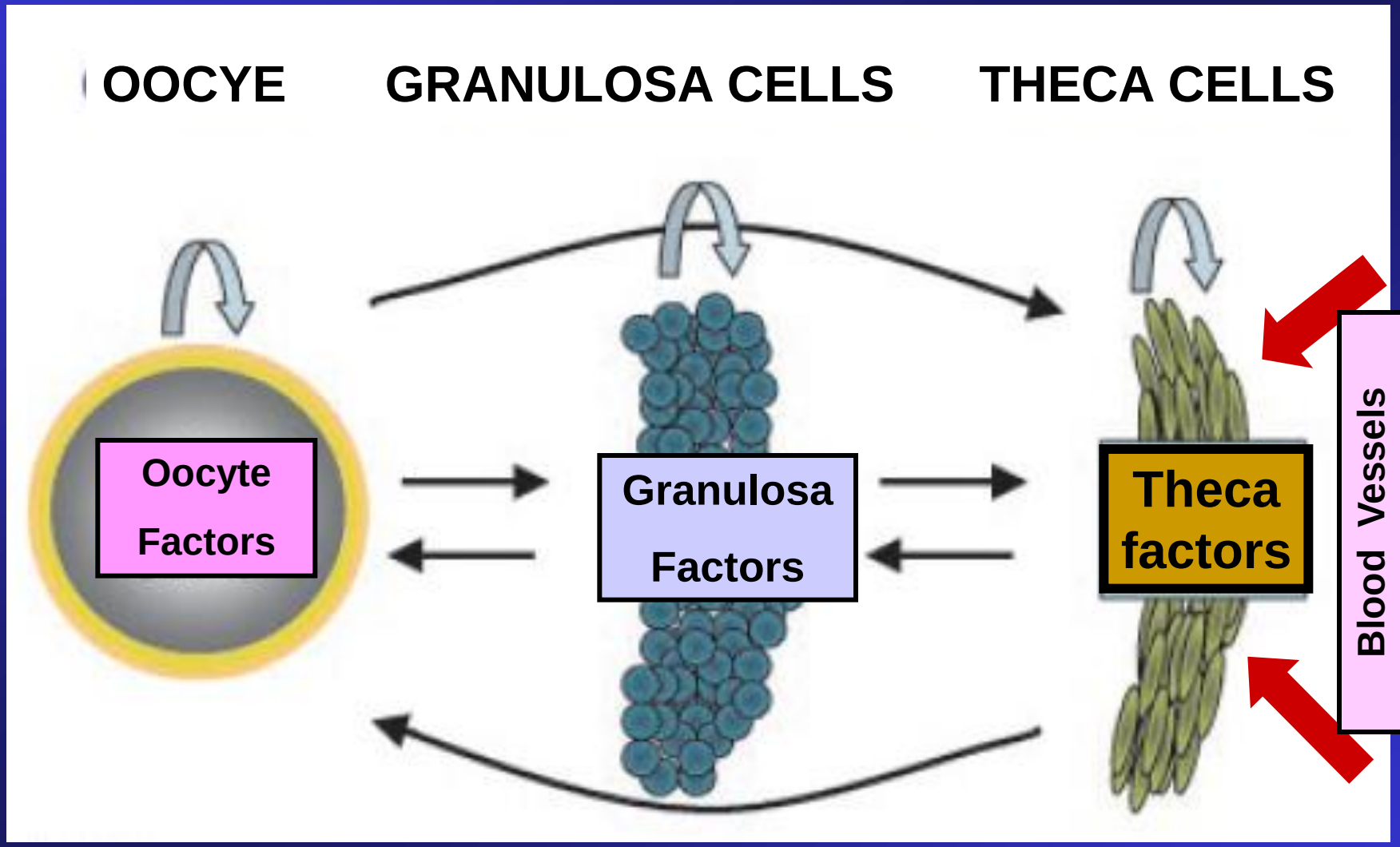
Background:

Possible Metabolic Mediators of Reproduction



From: Spicer, 2001 (Dom Anim Endocr 21:251-270)

Endocrine- Autocrine- Paracrine Action of Factors in Ovarian Follicles



Modified from: Shimasaki et al., 2004 (Endocr. Rev. 25:72-101)

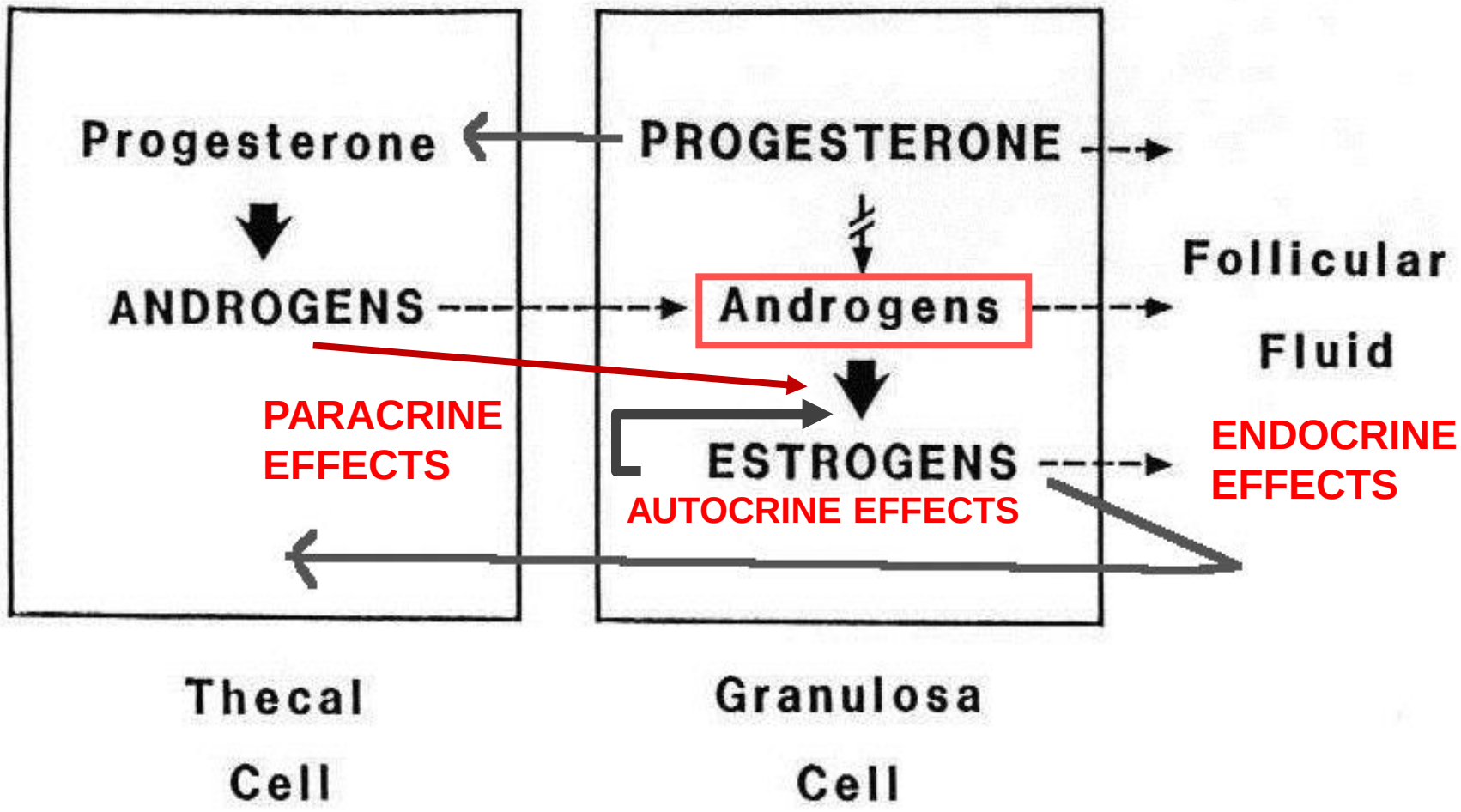
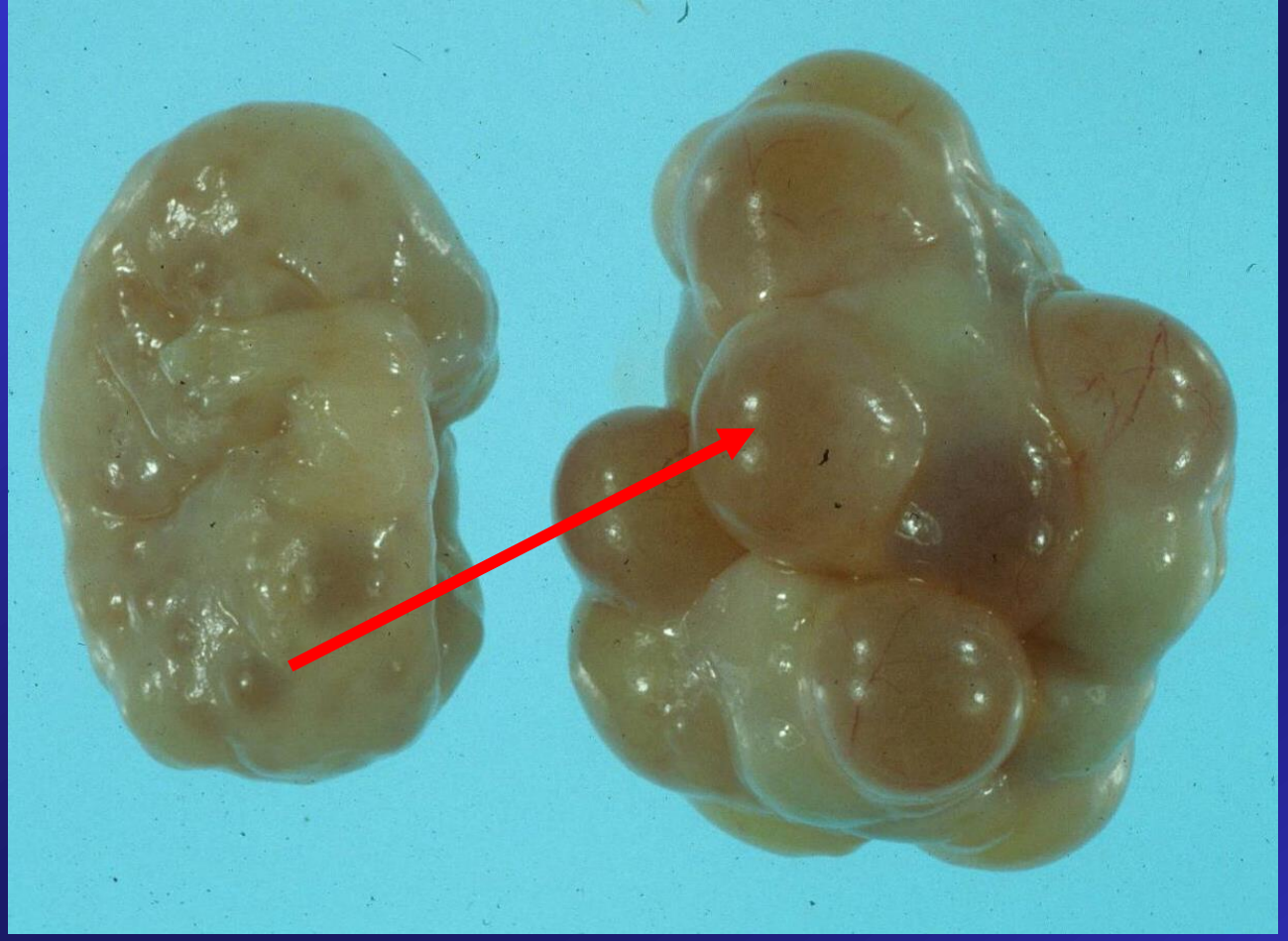
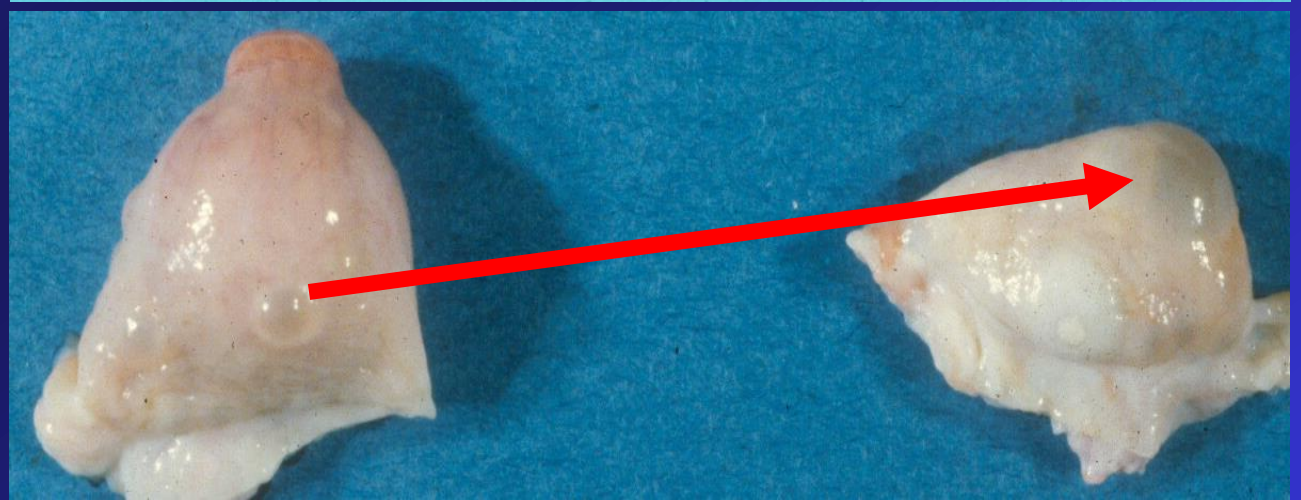
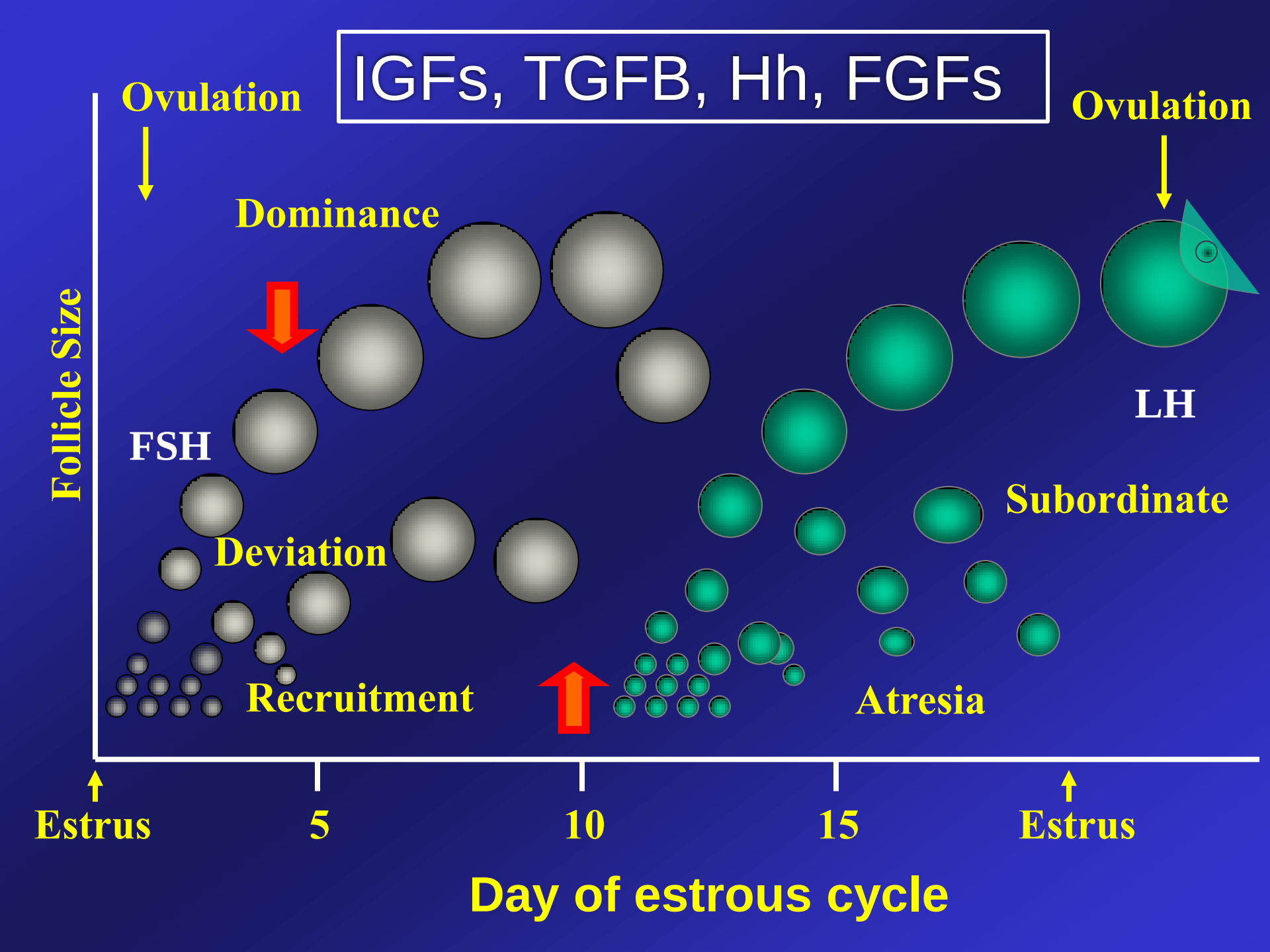


Figure 2—Schematic of the steroidogenic pathway depicting the cellular source of progesterone, androgens, and estrogens found in follicular fluid.

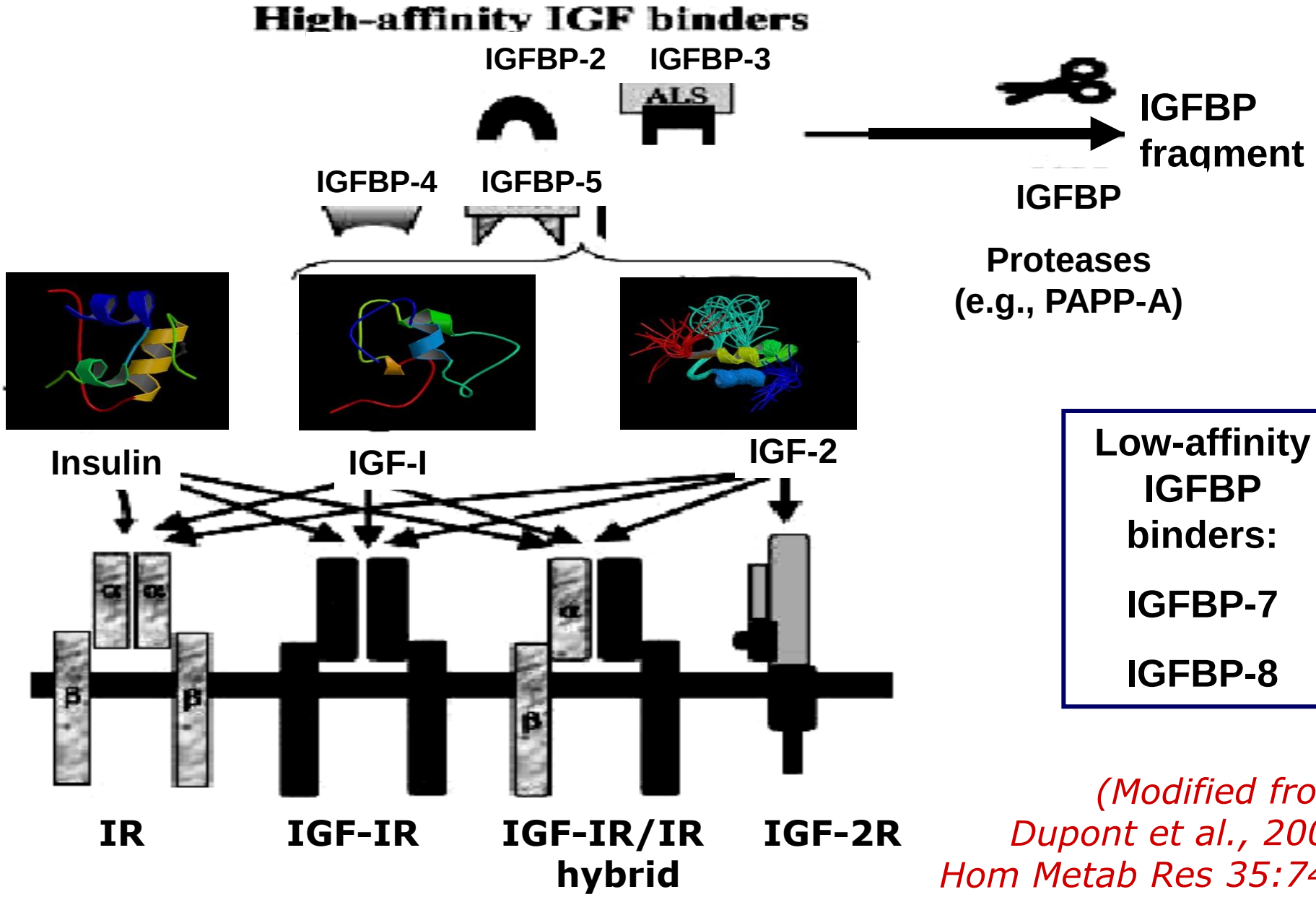
Normal Ovarian Follicle Growth






IGFs, TGFB, Hh, FGFs
Ovulation
Ovulation
Dominance
FSH
LH
Subordinate
Deviation
Recruitment
Atresia
Follicle Size
Estrus
5
10
15
Estrus
Day of estrous cycle

The complexity of the IGF system



Low-affinity IGFBP binders:

IGFBP-7

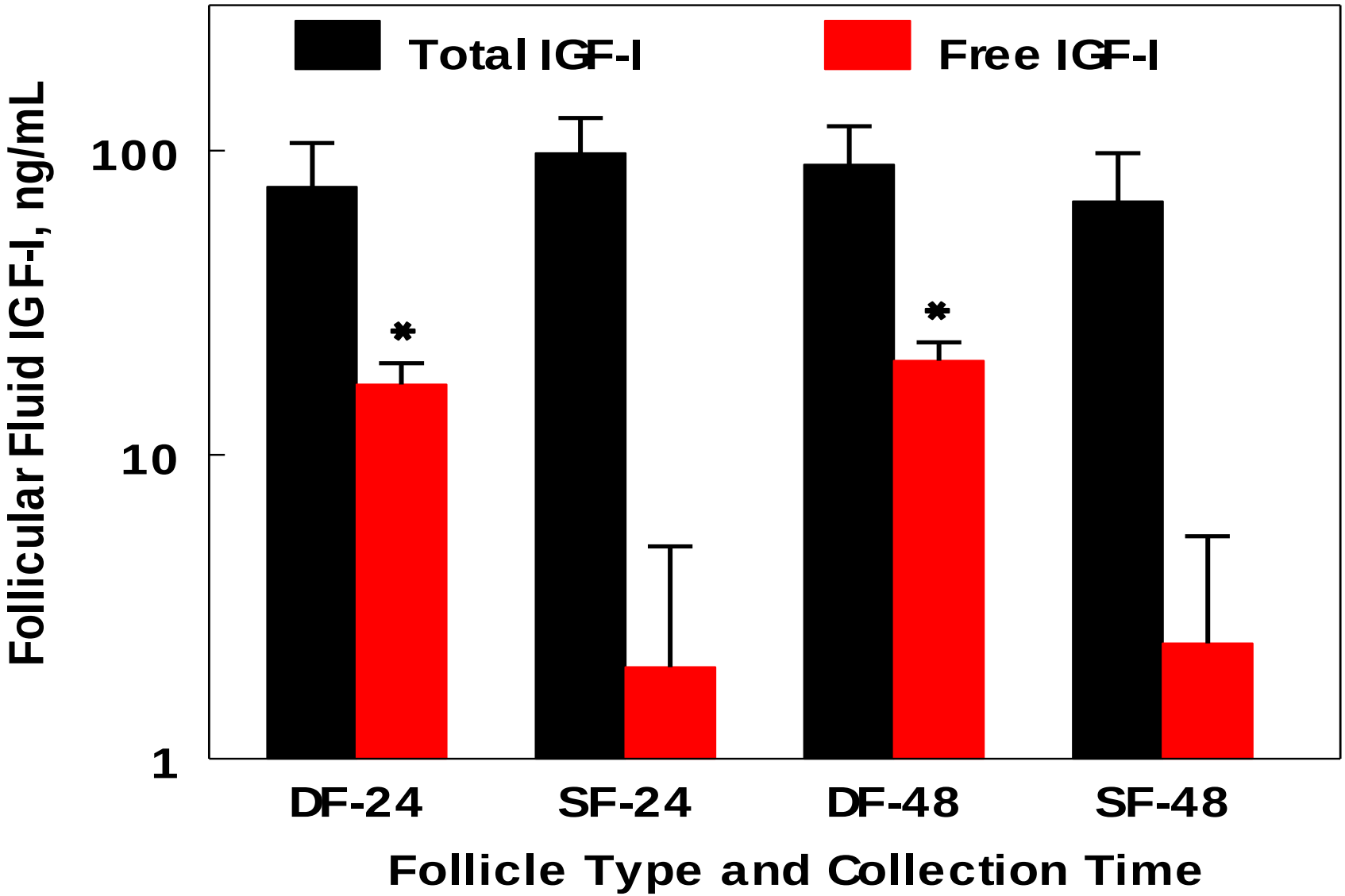
IGFBP-8

(Modified from: Dupont et al., 2003; Hom Metab Res 35:740)

During Follicular Growth:

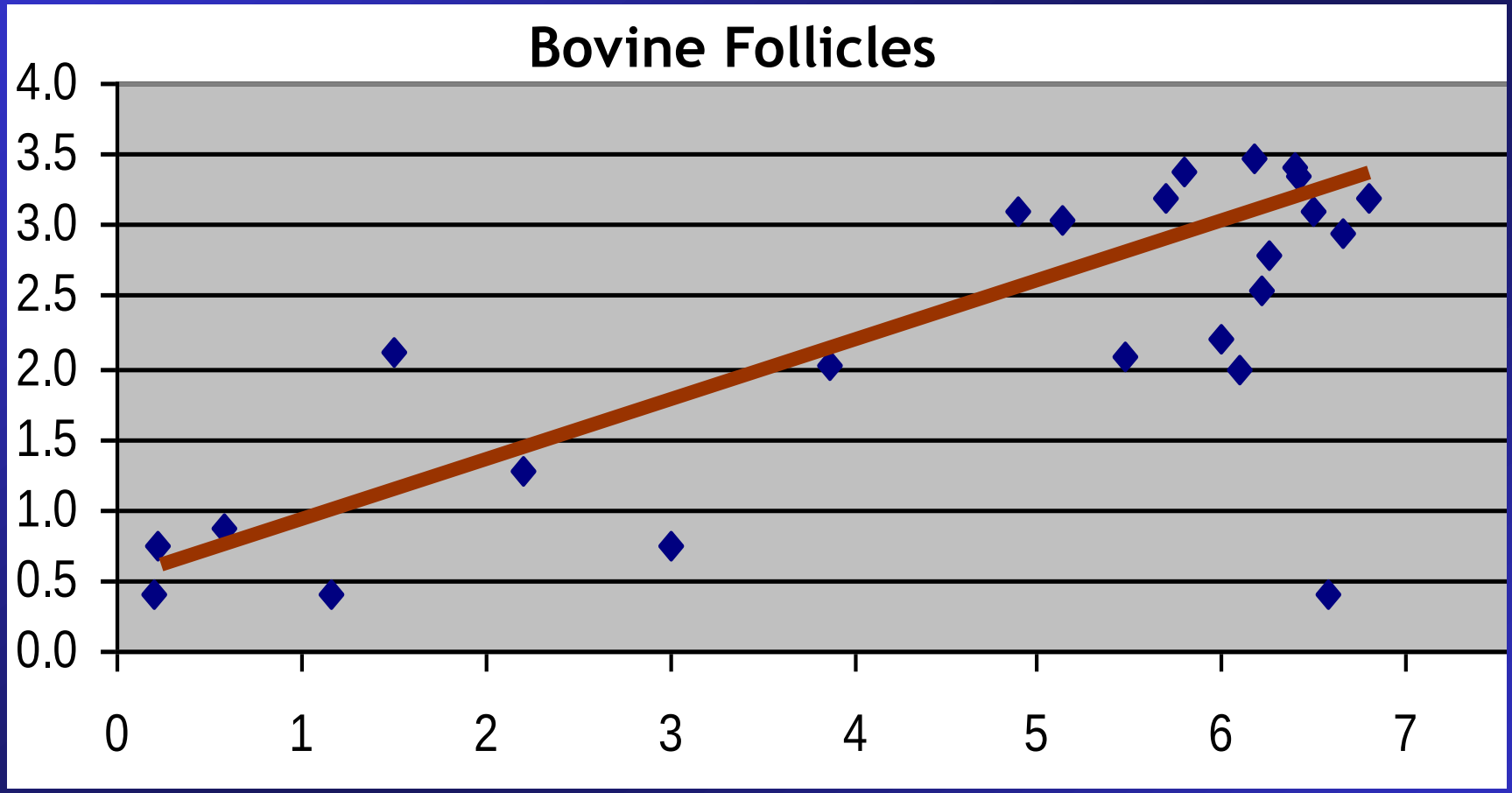
- **Estradiol and Androstenedione**
  - **levels in follicular fluid (FFL) are greater in Dominant vs. Subordinate follicles.**
- **IGF-I**
  - **Levels (total) in FFL do not differ between Dominant and Subordinate follicles – FREE concentrations are greater.**
  - **Primarily endocrine in origin.**
  - **stimulates steroidogenesis and mitogenesis of ovarian cells.**

Free IGF-I: DF > SF



(Santiago et al., 2005; Domest Anim Endocr 28:48-63)

Correlation of Estradiol & Free IGF-I

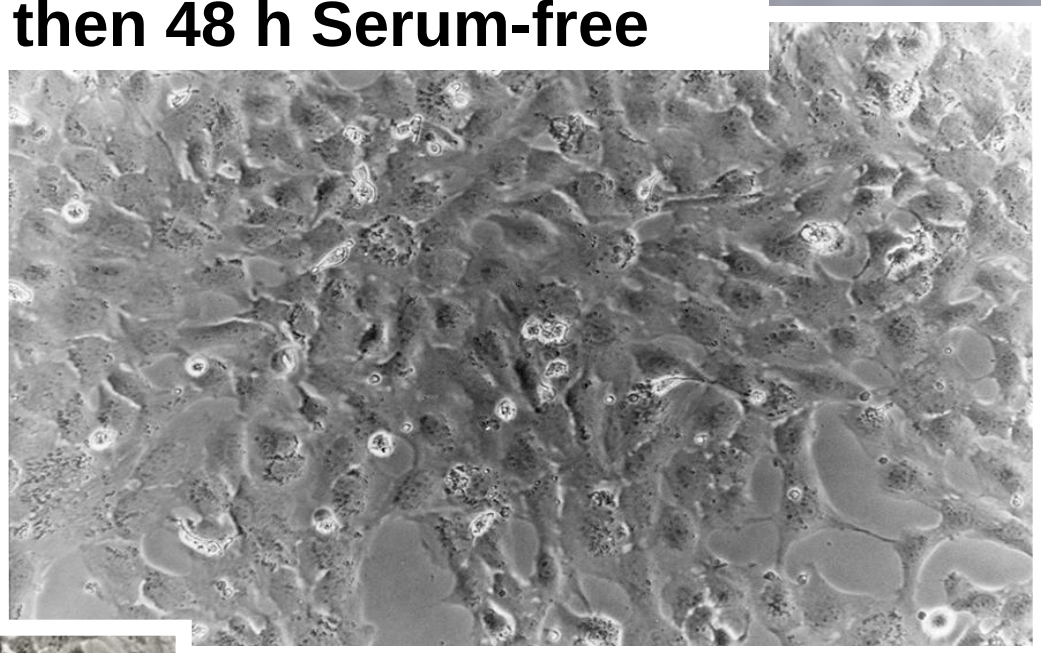


Free IGF-I positively correlated ($P<0.001$) with E_2 ($r=0.73$)

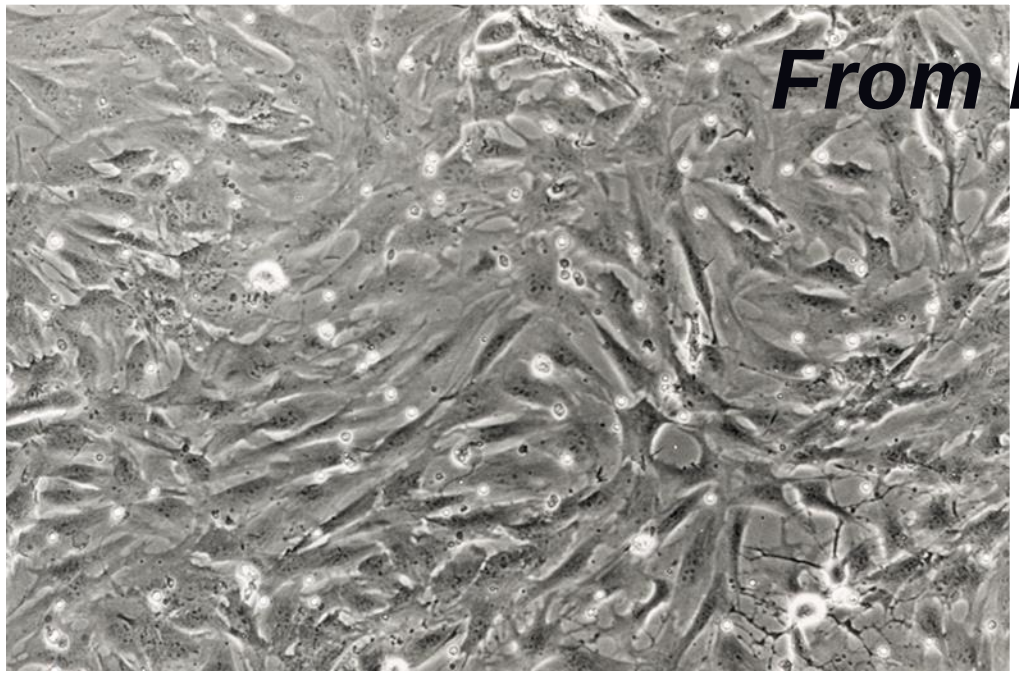
(Santiago et al., 2005; Domest Anim Endocr 28:48-63)

48 h 10% FCS then 48 h Serum-free

Cultured Granulosa Cells →

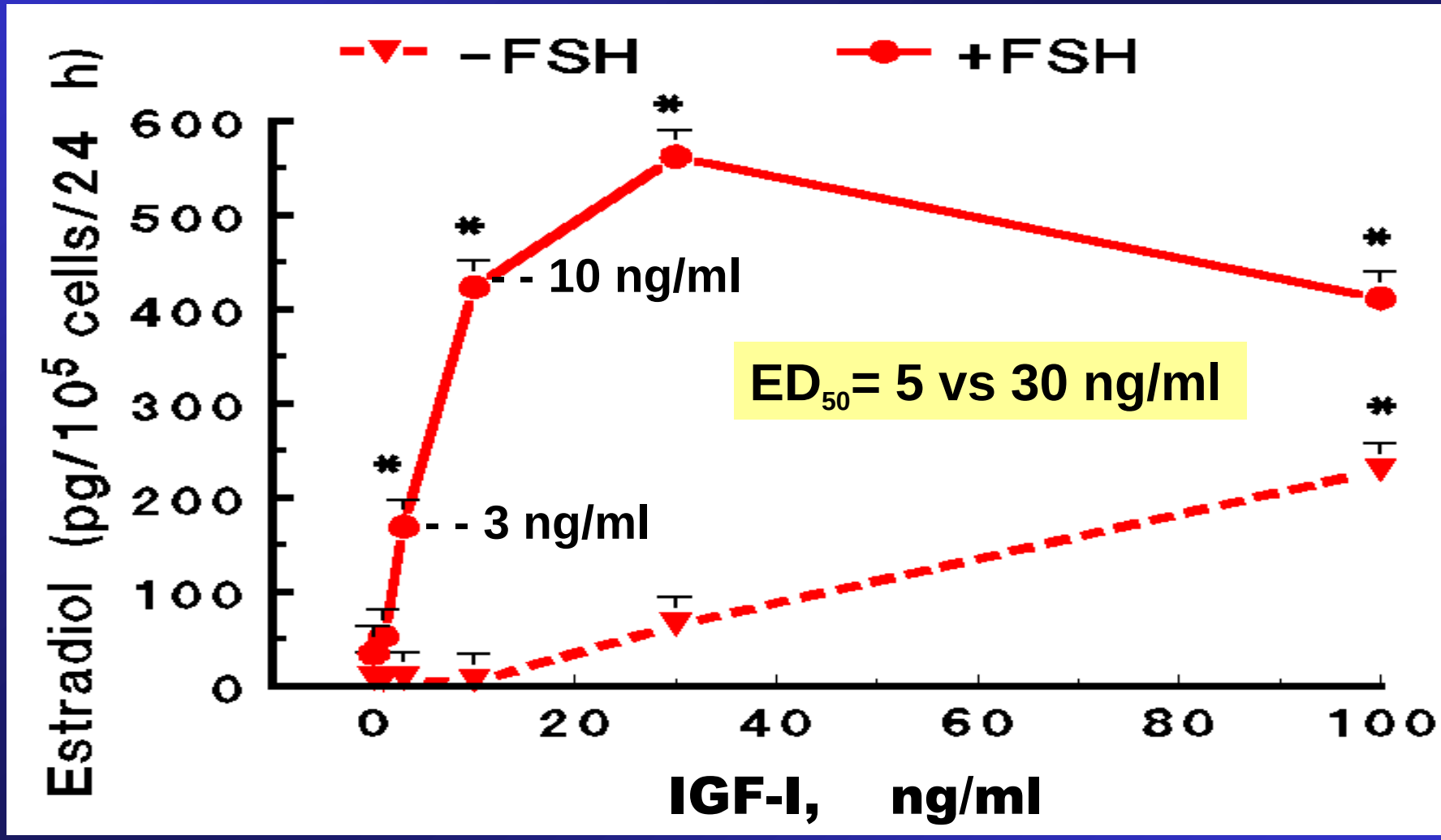


From bovine follicles



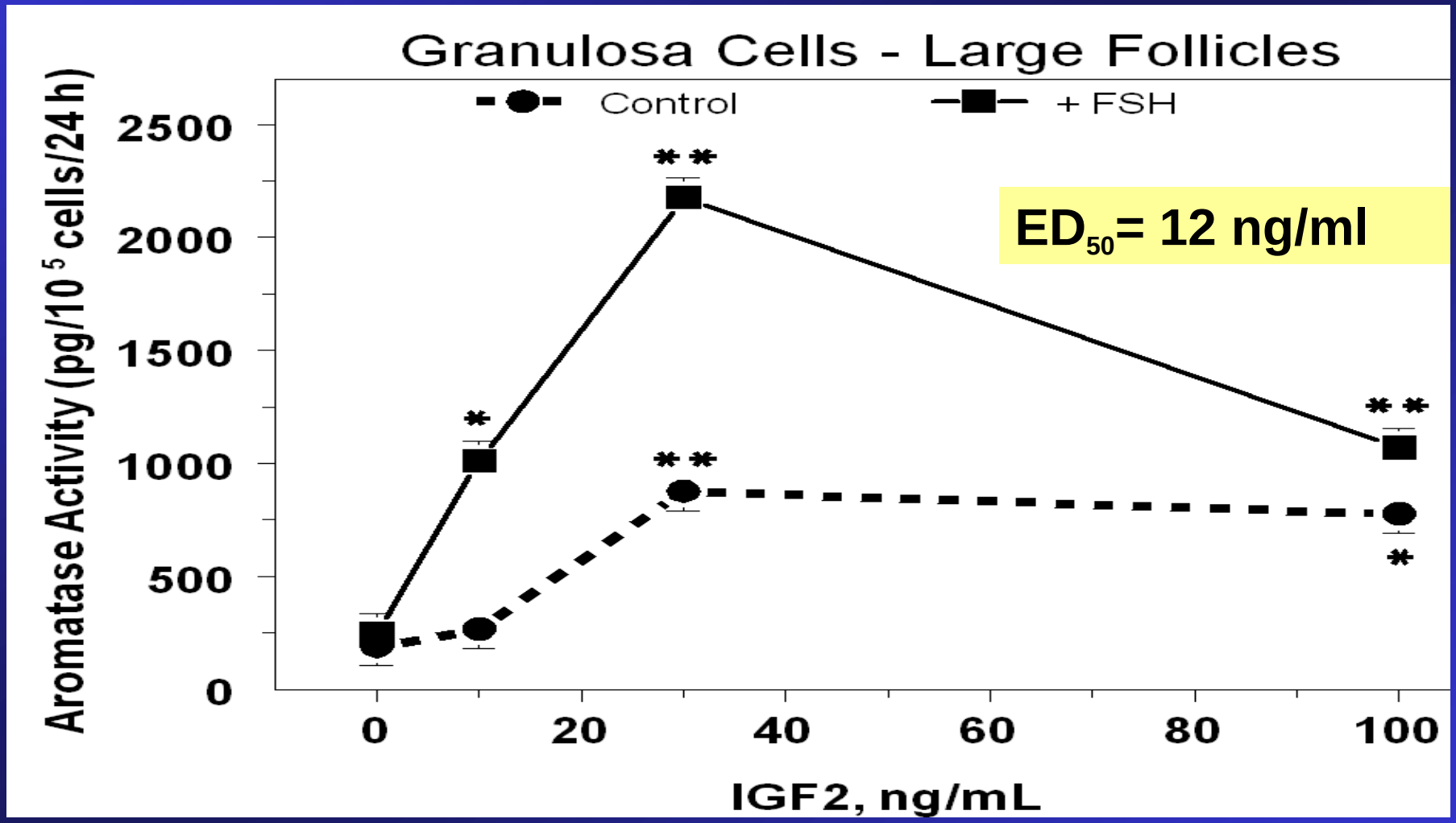
← Cultured Thecal Cells

IGF-I Stimulates Aromatase Activity in Bovine Granulosa Cells –Small Follicles



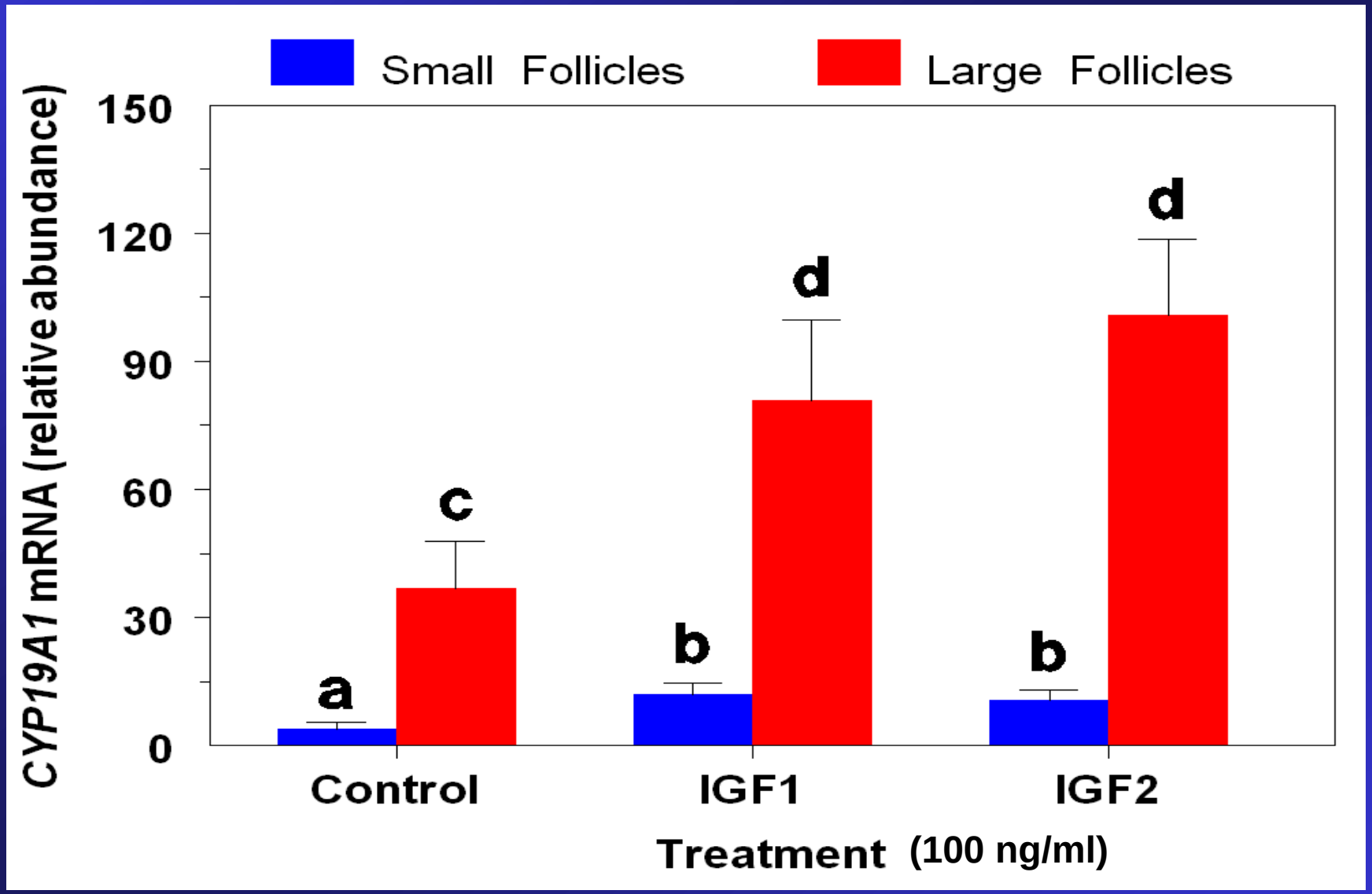
Spicer et al., 2002 (Domest Anim Endocr 22:237-54)

IGF-2 Stimulates Aromatase Activity in Bovine Granulosa Cells – Large Follicles



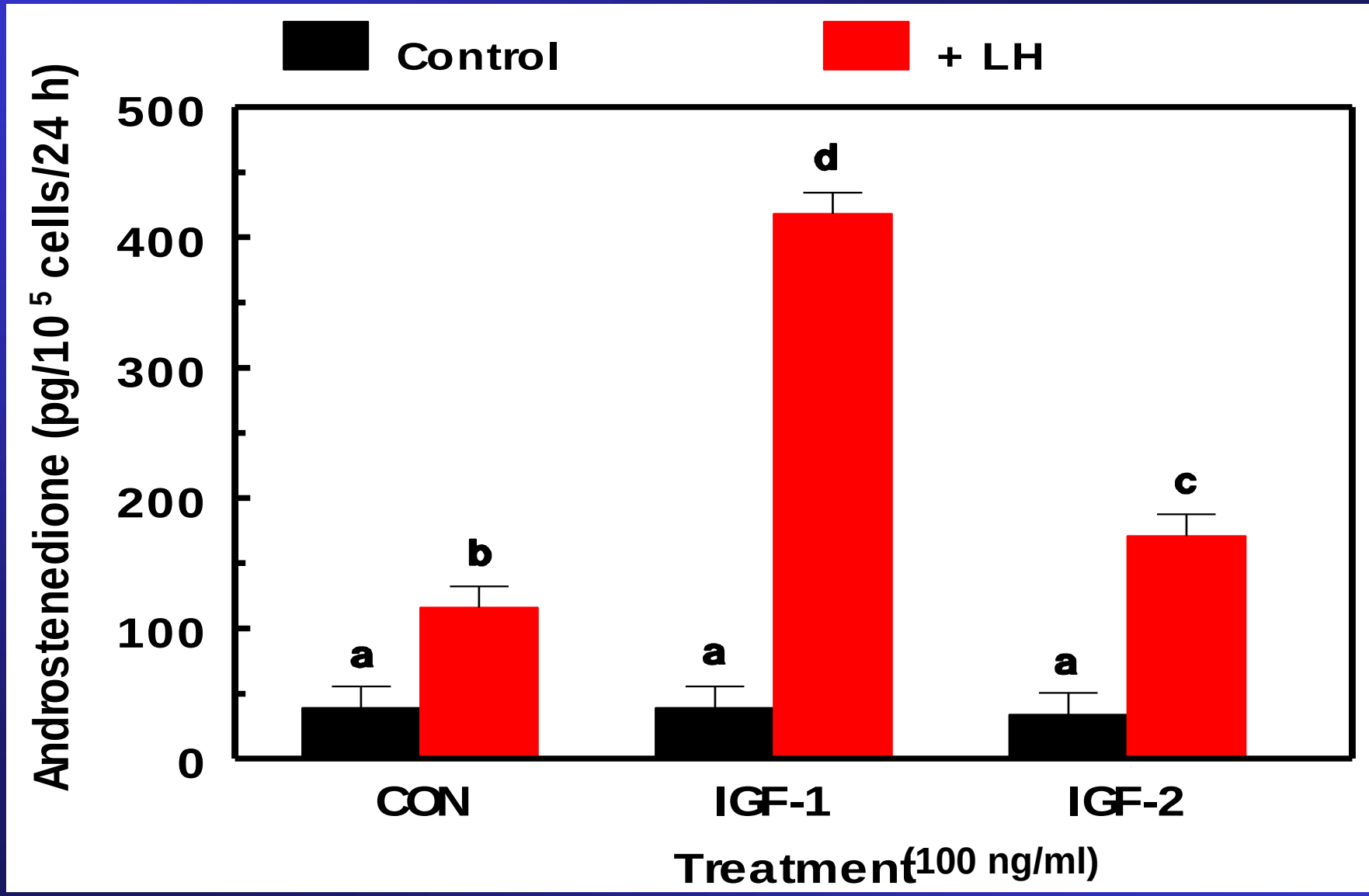
(Spicer & Aad, 2007; Biol. Reprod. 77:18-27)

IGFs Stimulates CYP19A1 in Granulosa Cells



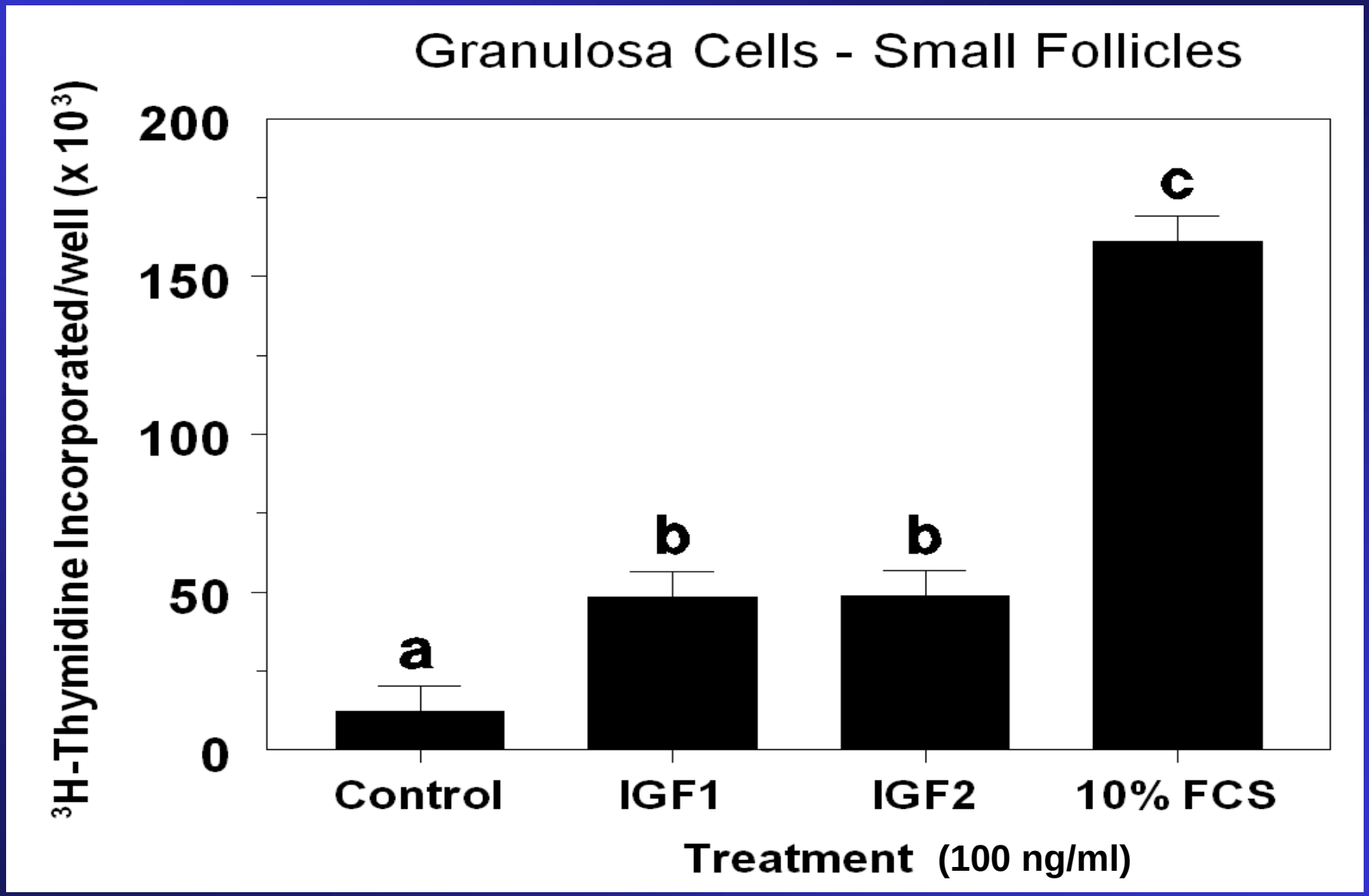
(Spicer & Aad, 2007; Biol. Reprod. 77:18-27)

IGFs Stimulates Steroidogenesis in Thecal Cells



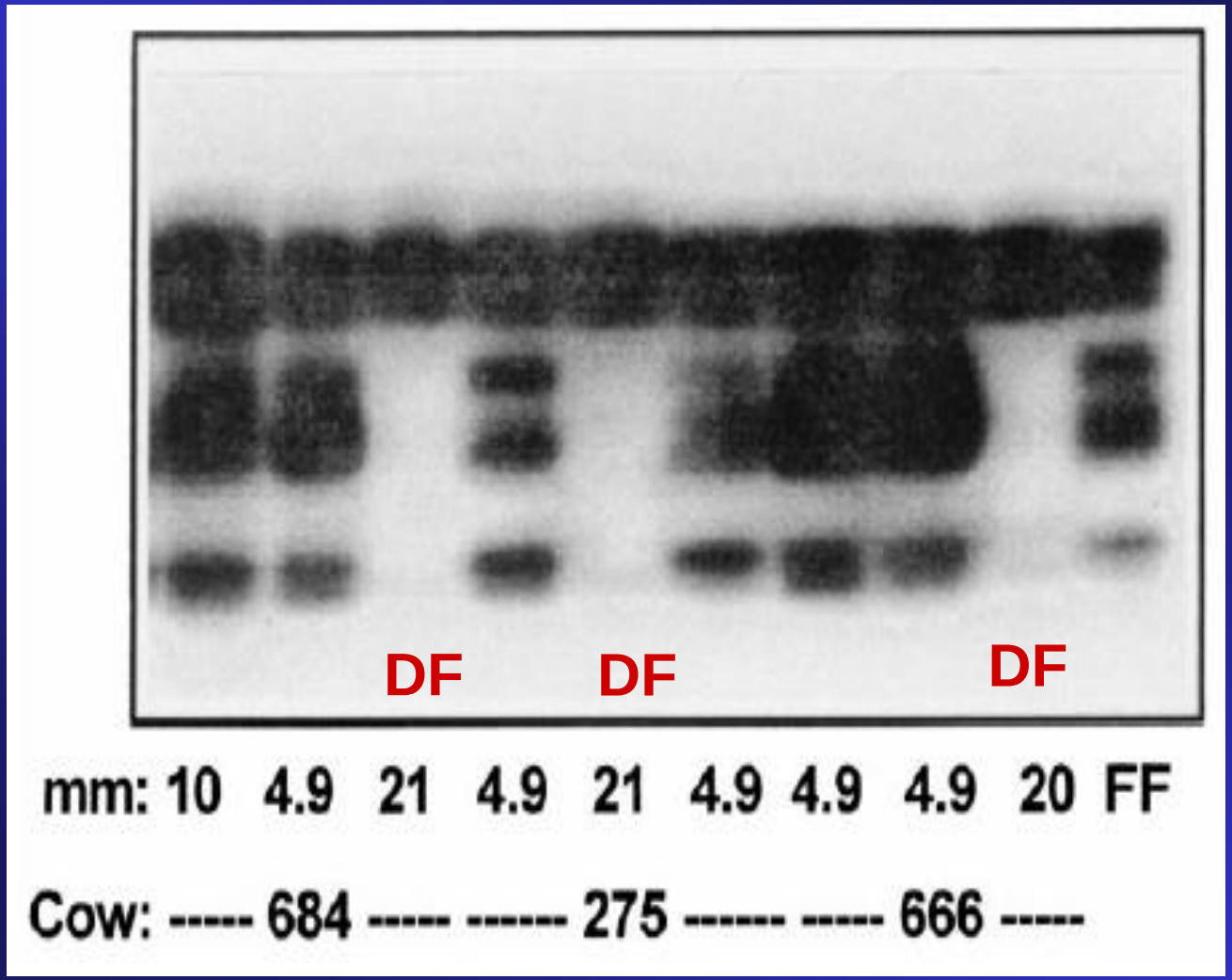
Spicer et al., 2004 (Mol. Cell. Endocr. 227:1-7)

IGFs Stimulates Proliferation of Granulosa Cells



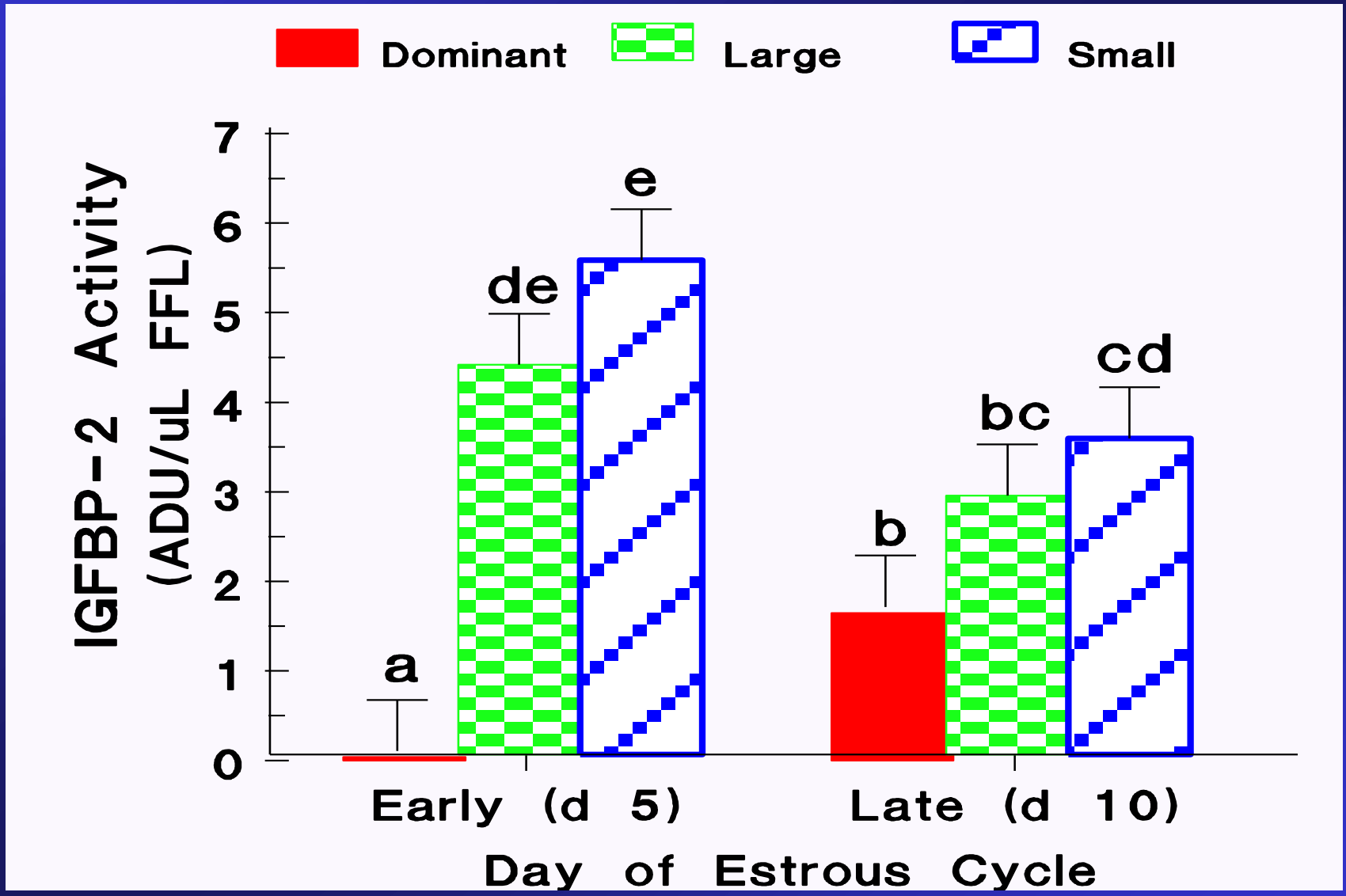
(Spicer & Aad, 2007; Biol. Reprod. 77:18-27)

IGFBPs in Dominant vs. Subordinate Bovine Follicles



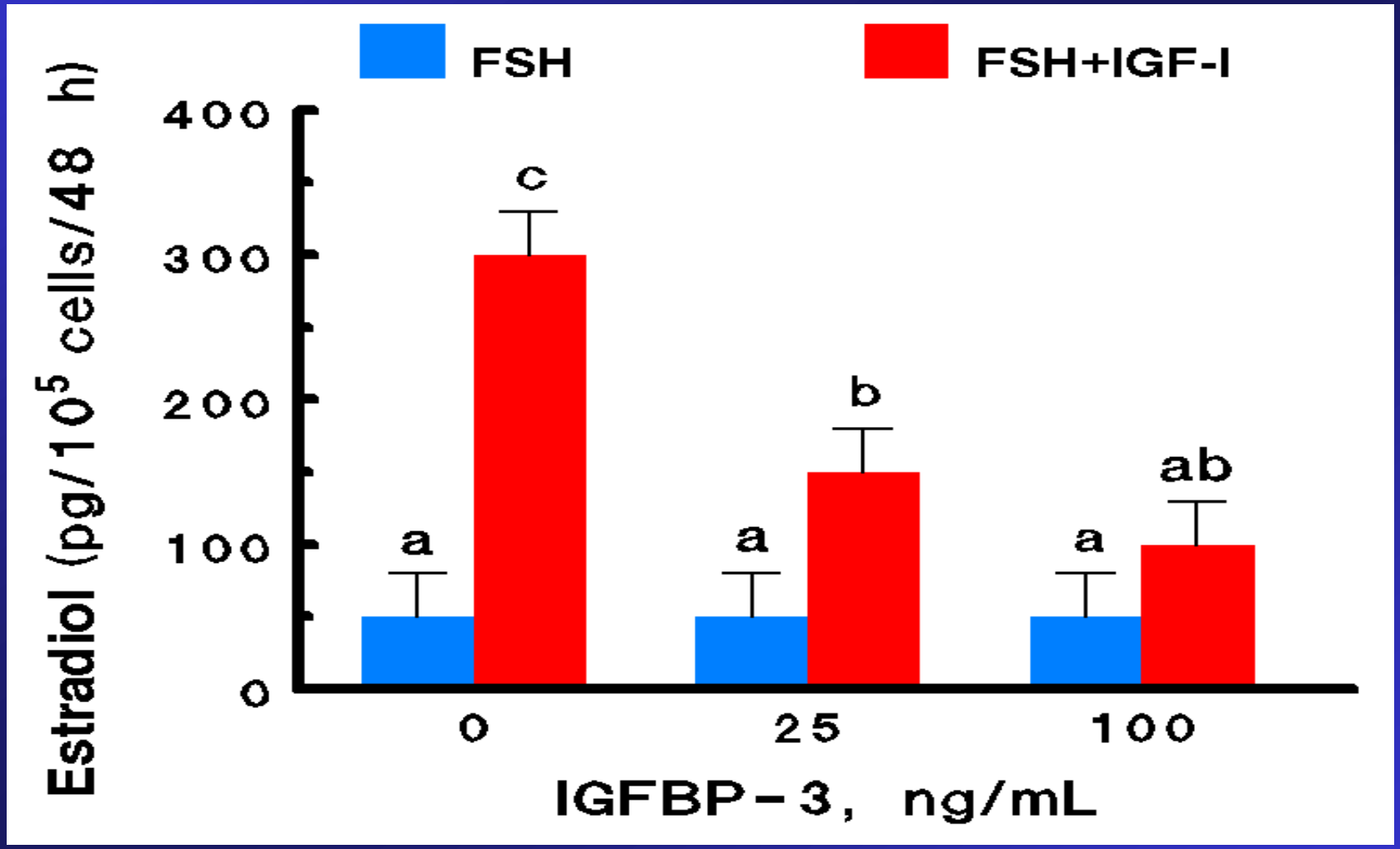
From Spicer et al., 2001 (Domest. Anim. Endocr. 21:1-15)

IGFBP-2 Activity is Lower in DF



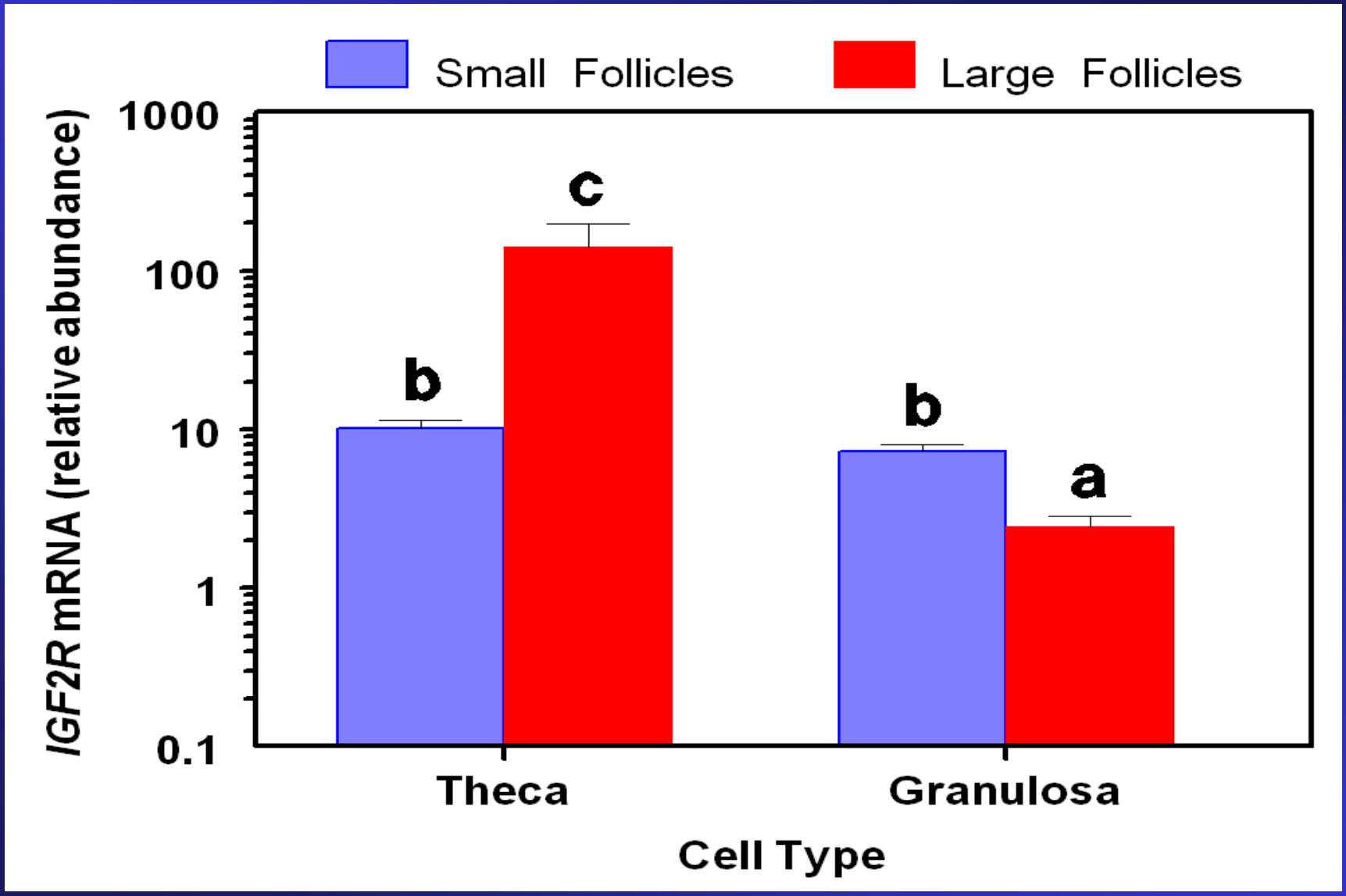
From Stewart et al., 1996 (Endocrinology 137:2842)

IGFBP-3 Inhibits IGF-I-induced Aromatase Activity by Granulosa Cells



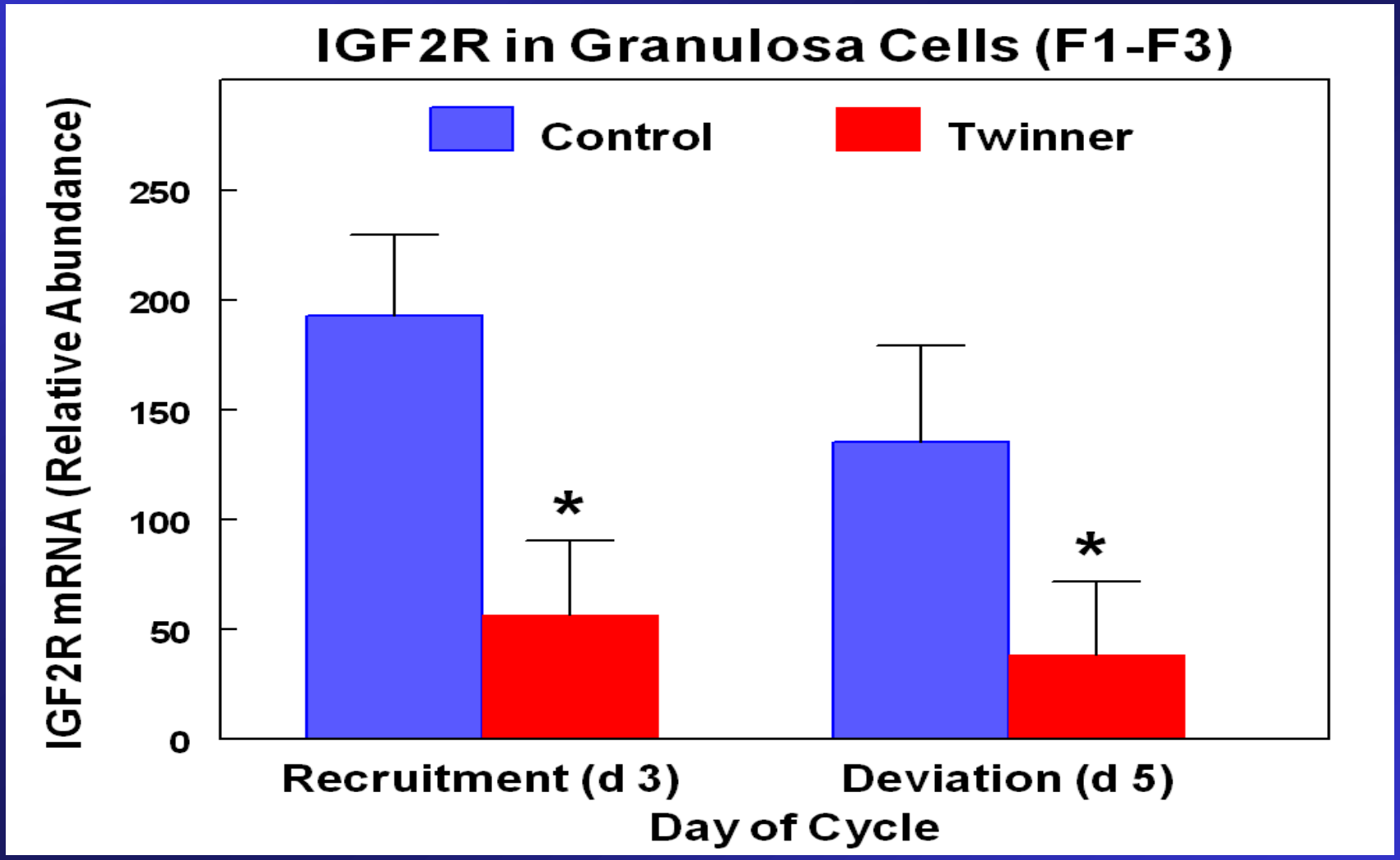
Spicer & Chamberlain, 1999 (Dom. Anim. Endocr. 16:19-29)

IGF2R decreases with increased follicle size



(Spicer & Aad, 2007; Biol. Reprod. 77:18-27)

IGF2Rc mRNA is decreased in Granulosa cells of Twinner cattle



(Modified from: Aad et al., 2013; Domest. Anim. Endocr. 45:187)

Summary:

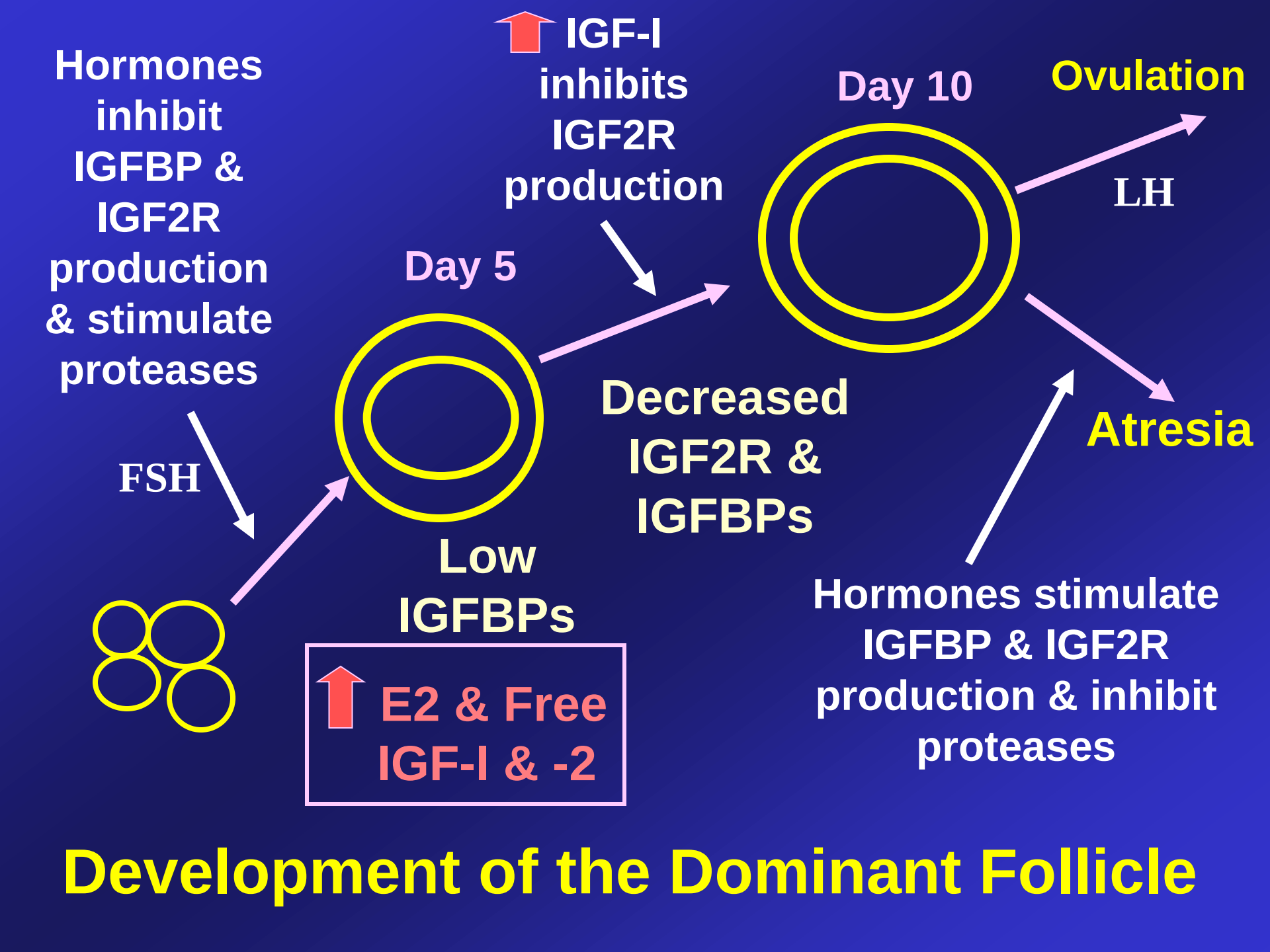
- **IGF-I & -2 stimulate steroid production and cell proliferation.**
- **IGFBPs block IGF binding and cell responses.**
- **IGF2Rc is reduced in co-dominant follicles**

During Follicular Growth:

- **IGFBPs**
  - **IGFBP-2, -4 and -5 levels decrease.**

    (which bind IGF-I and –2 and block IGF action)

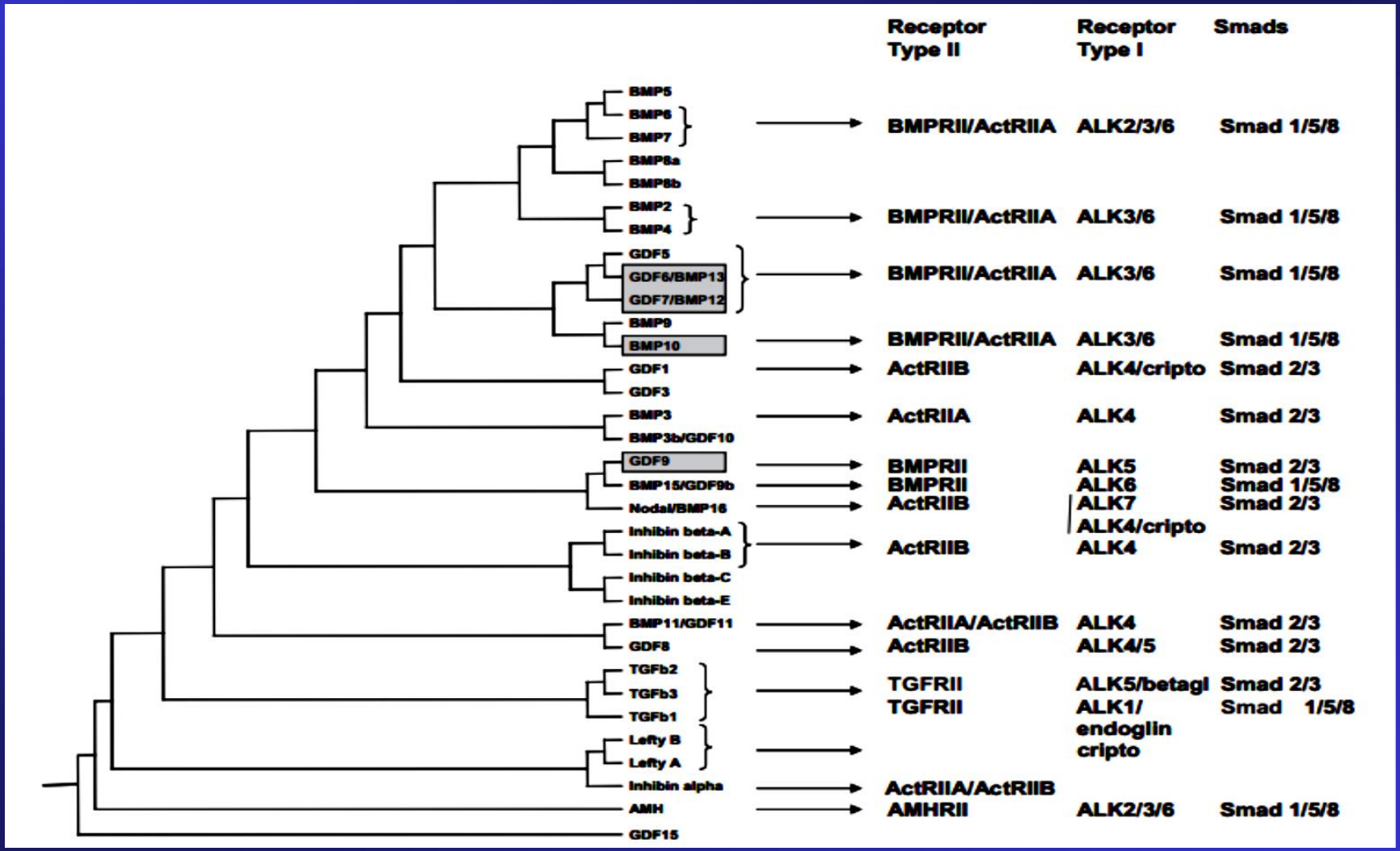
    thus, decreased IGFBPs cause increased "free" IGF which further stimulate follicle development.



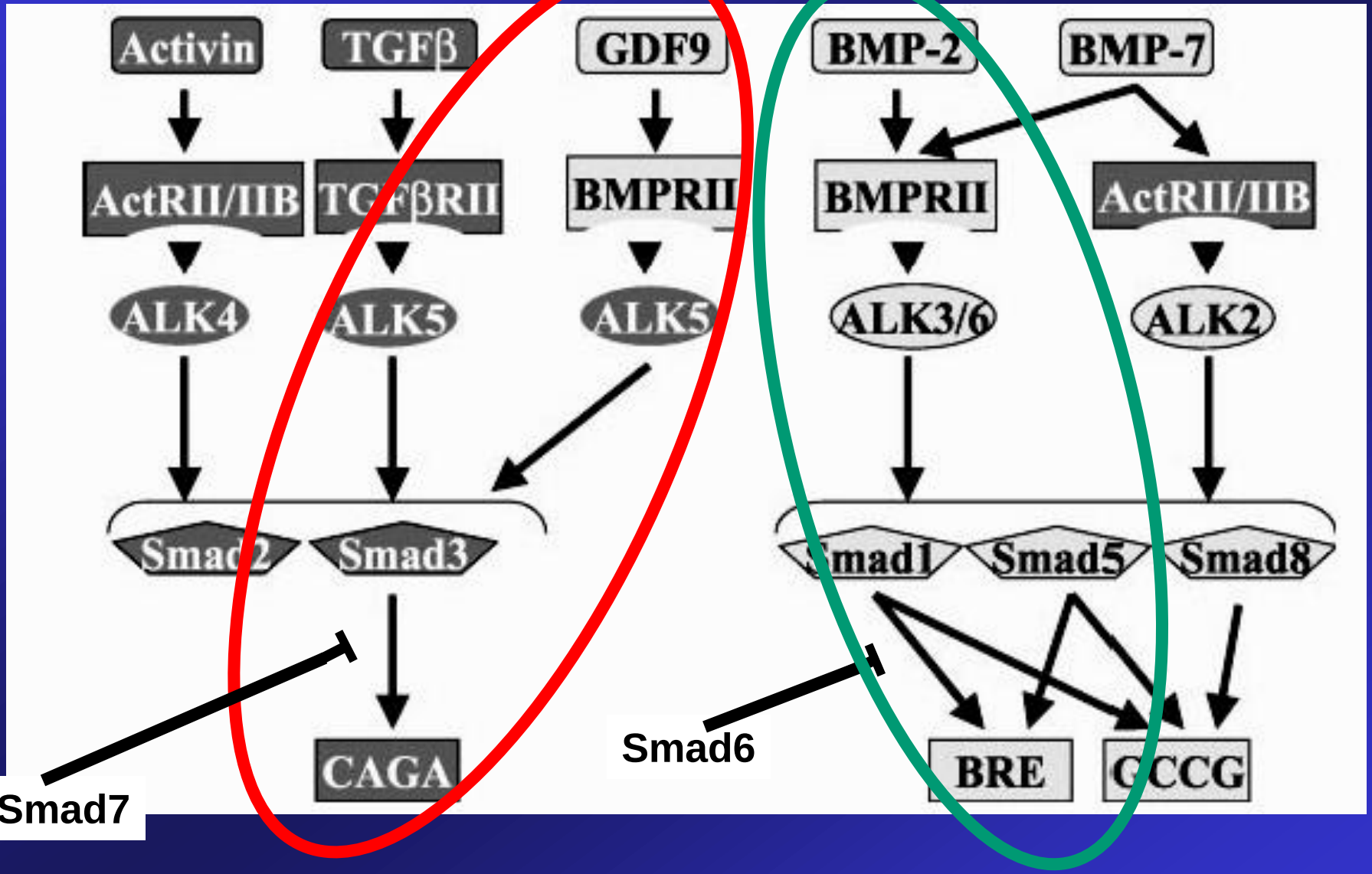
Development of the Dominant Follicle

Impact of TGFB family on Granulosa and Thecal Cell Steroidogenesis

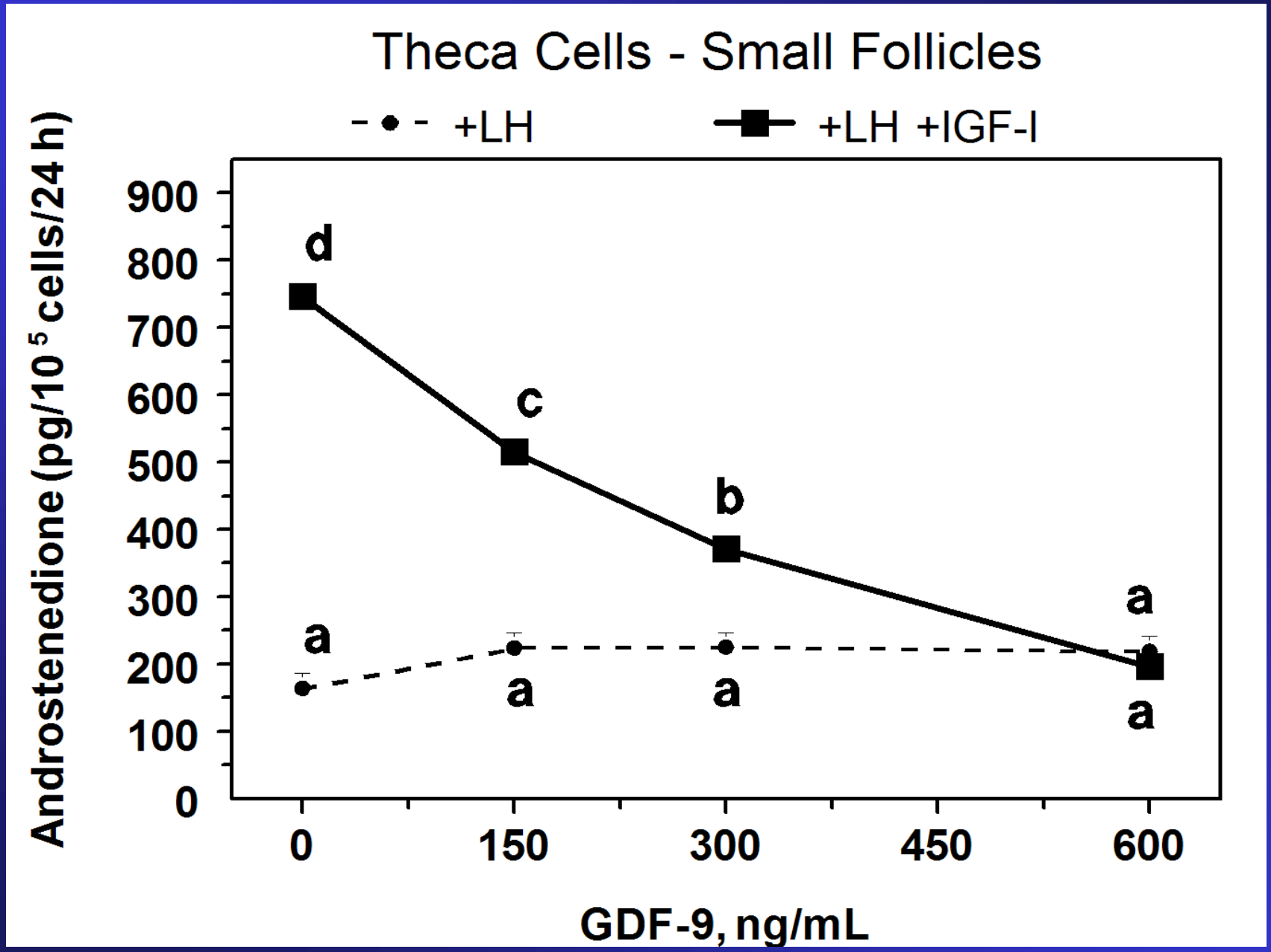
TGFB Family of Proteins



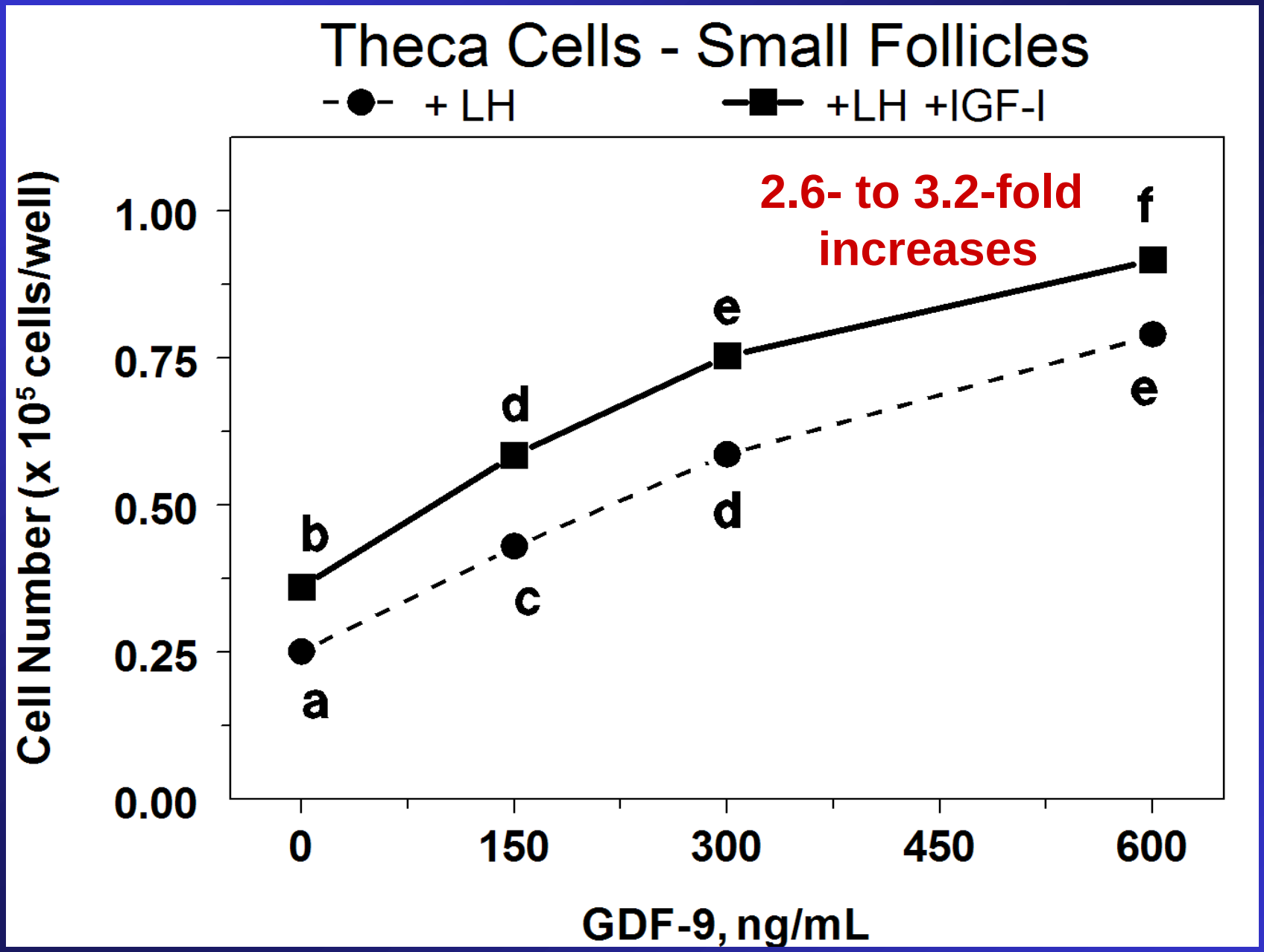
From: Mazerbourg & Hsueh, 2006 (Hum. Reprod. Update 12:373-83)



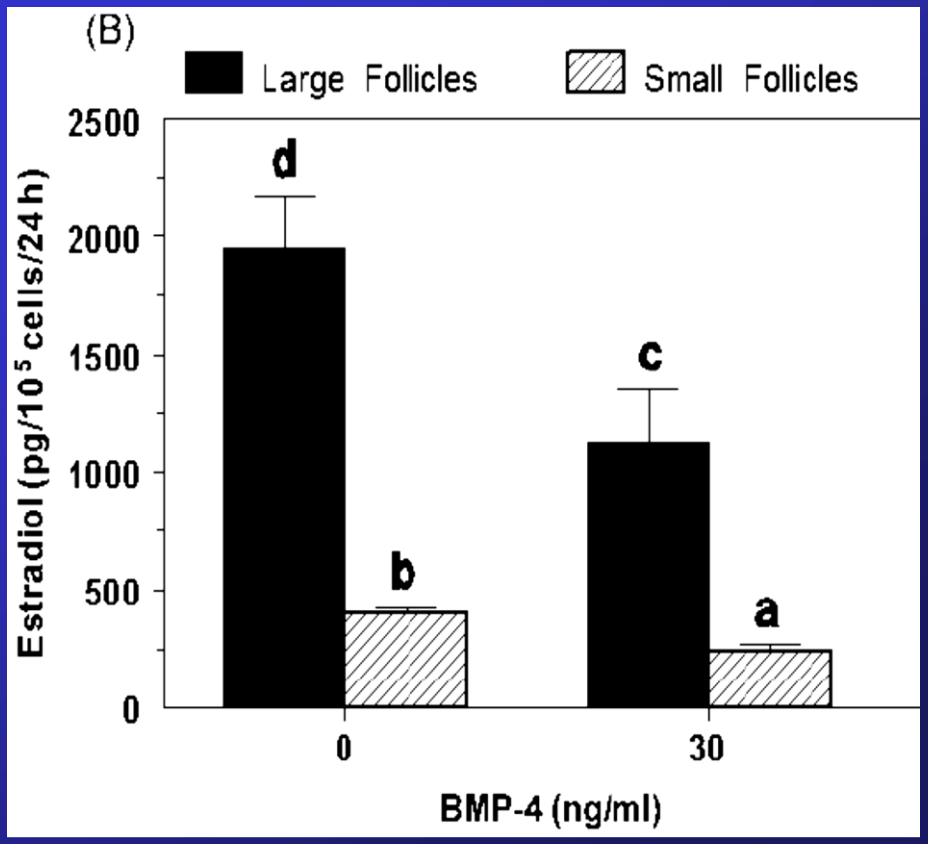
Intracellular signaling of GDF-9 vs. BMP-2/4



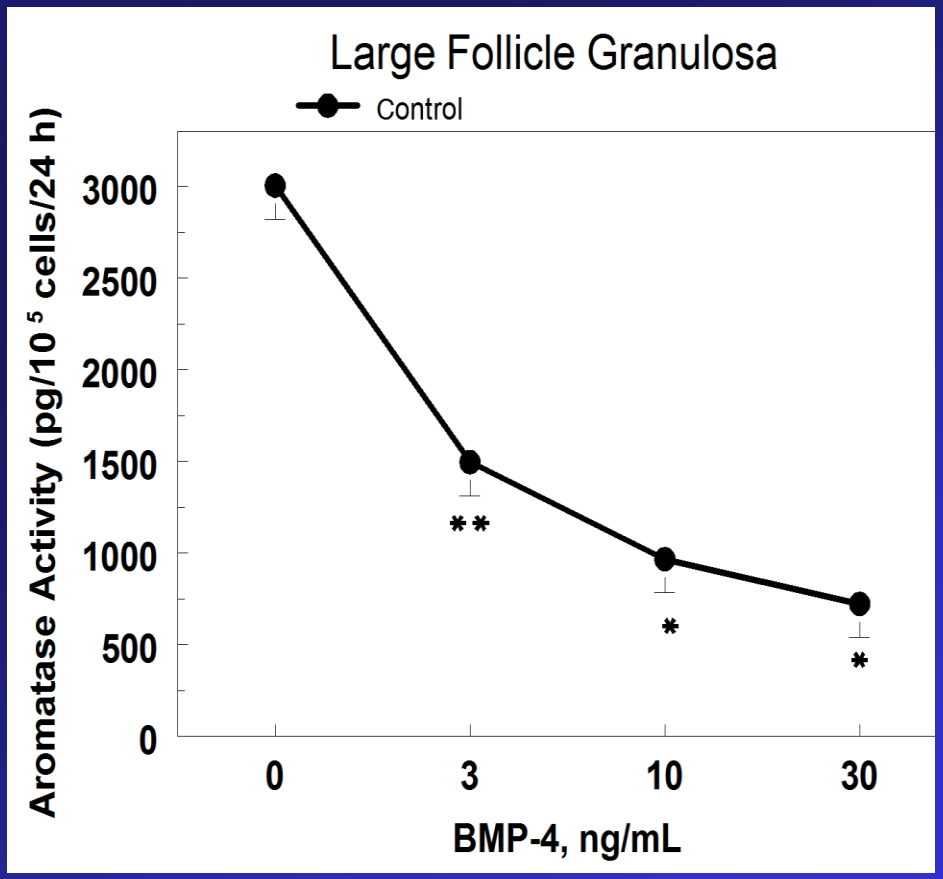
Redrawn from: Spicer et al., 2008 (Biol. Reprod. 78:243)

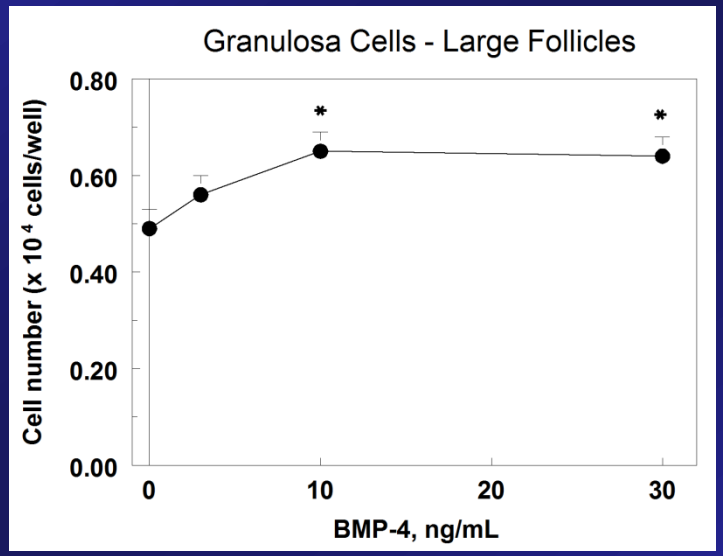


Redrawn from: Spicer et al., 2008 (Biol. Reprod. 78:243)



From: Spicer et al., 2006 (J. Endocrinol. 189:329)





Spicer et al., 2015 (unpublished)

Summary:

- **GDF9 stimulates cell proliferation and inhibits gonadotropin-induced steroid production in granulosa & theca cells (Spicer et al., 2006; 2008).**

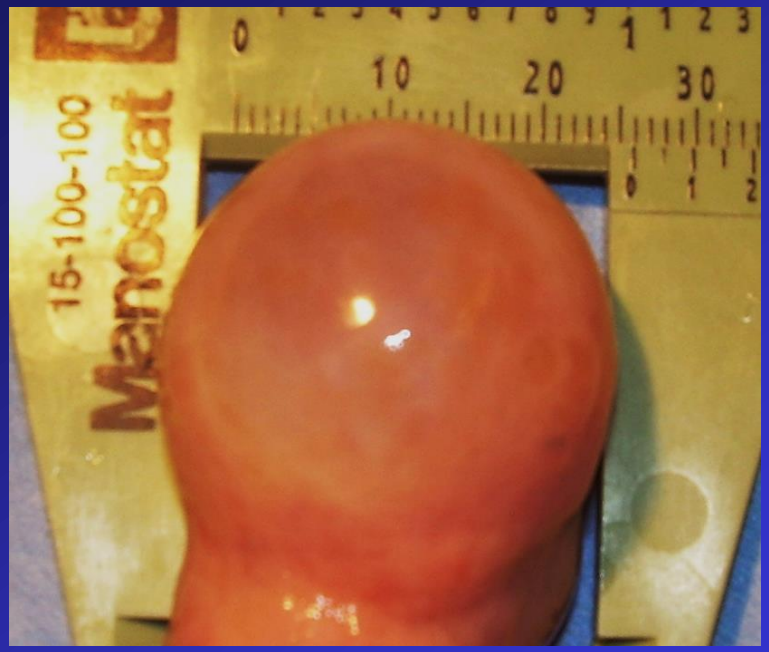
- **BMP4 is a potent inhibitor of steroidogenesis in granulosa and theca cells & stimulates granulosa cell proliferation.**

- **Microarray gene expression comparisons may aid in understanding additional causes of anovulation and ovarian follicular cyst formation.**

23,000 gene inquiries

VS.

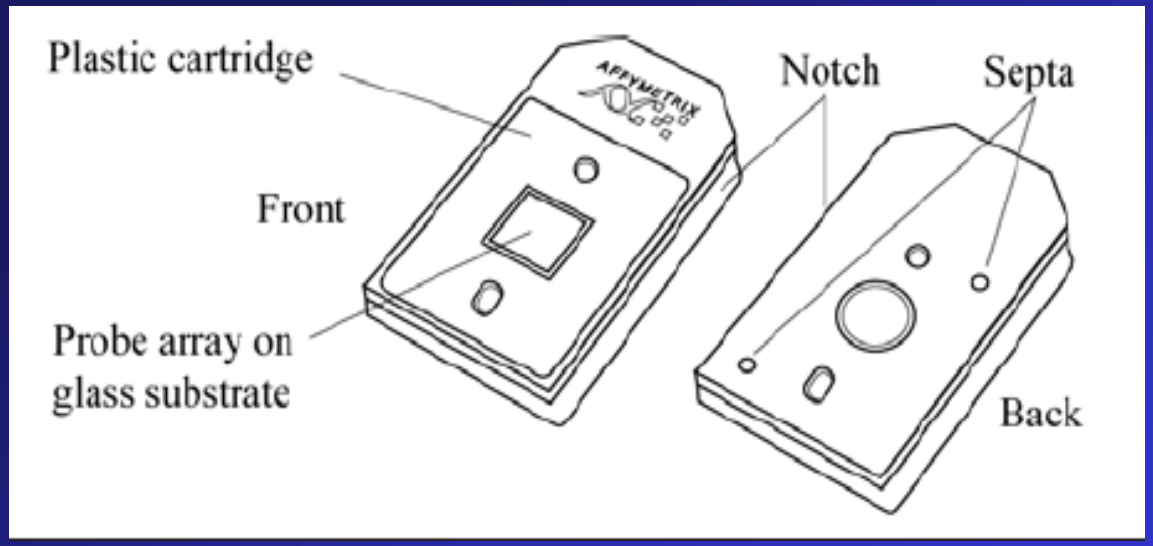
Hand full of candidate genes



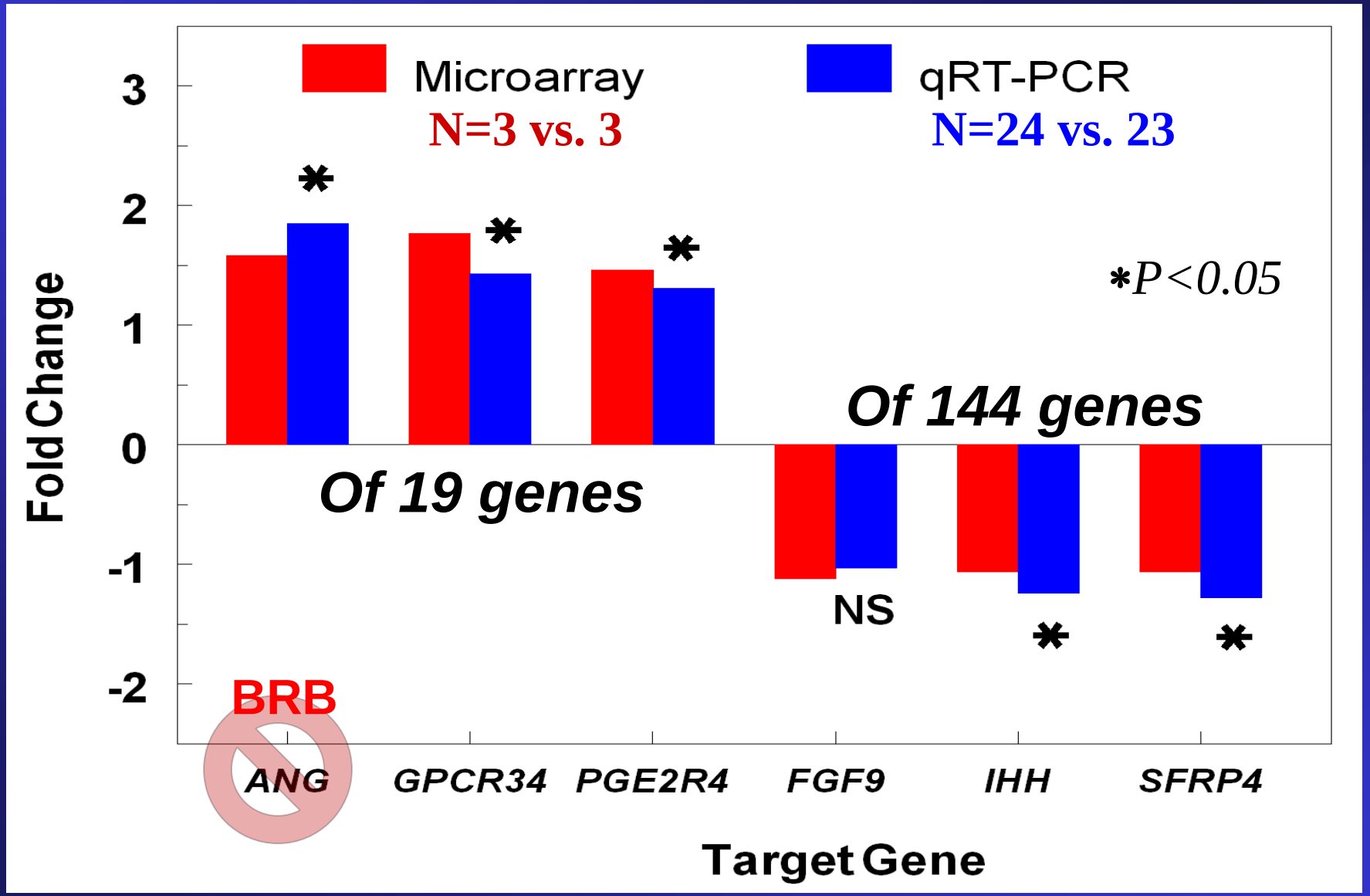
Microarray Analysis:

- Affymetrix Bovine GeneChips®
- 24,072 probe sets for Interrogation of approximately 23,000 bovine transcripts.

N = 163 genes
P < 0.01

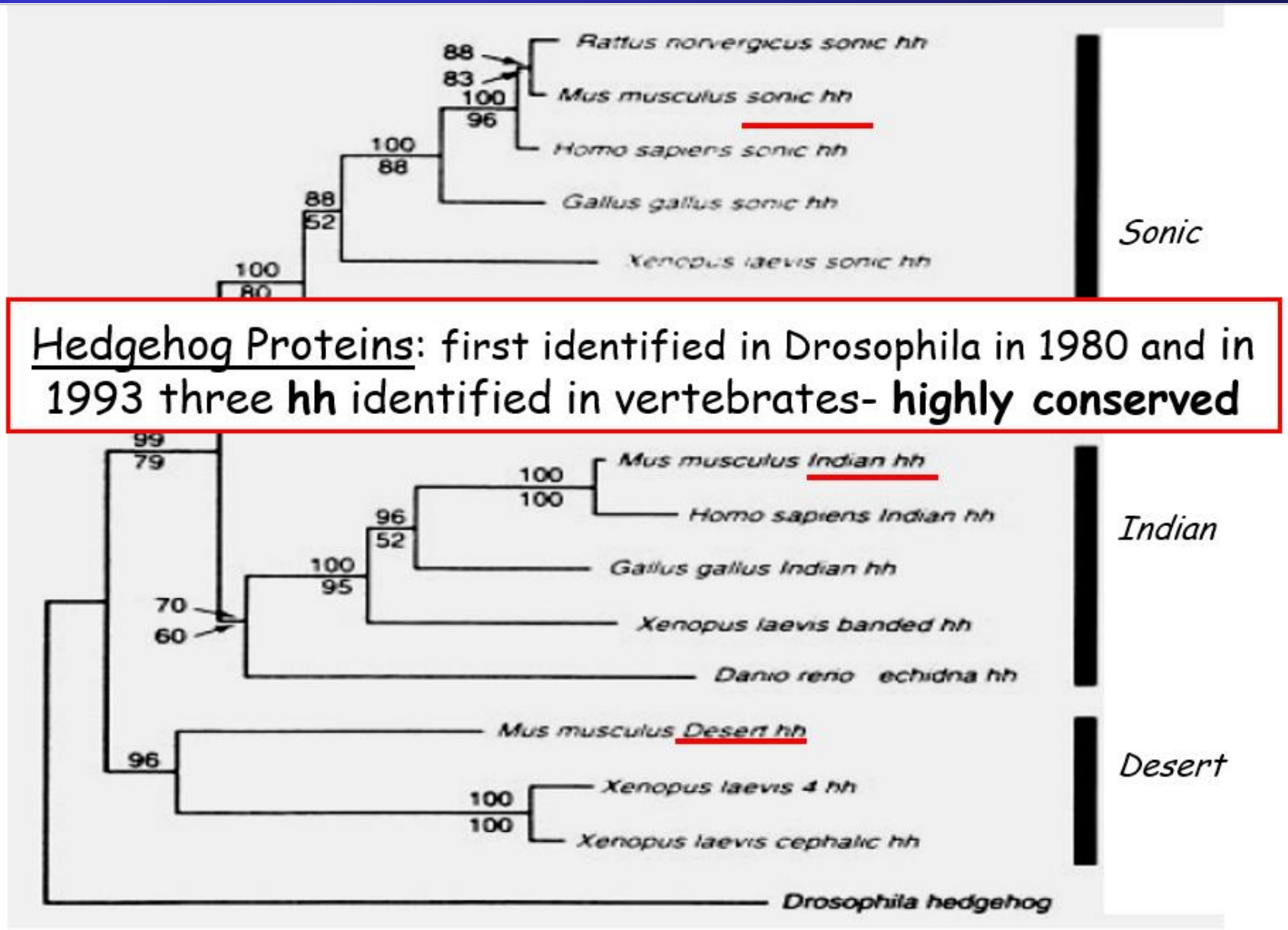


Target genes: Comparison of fold changes estimated through MA and RT-PCR (after log transformation):

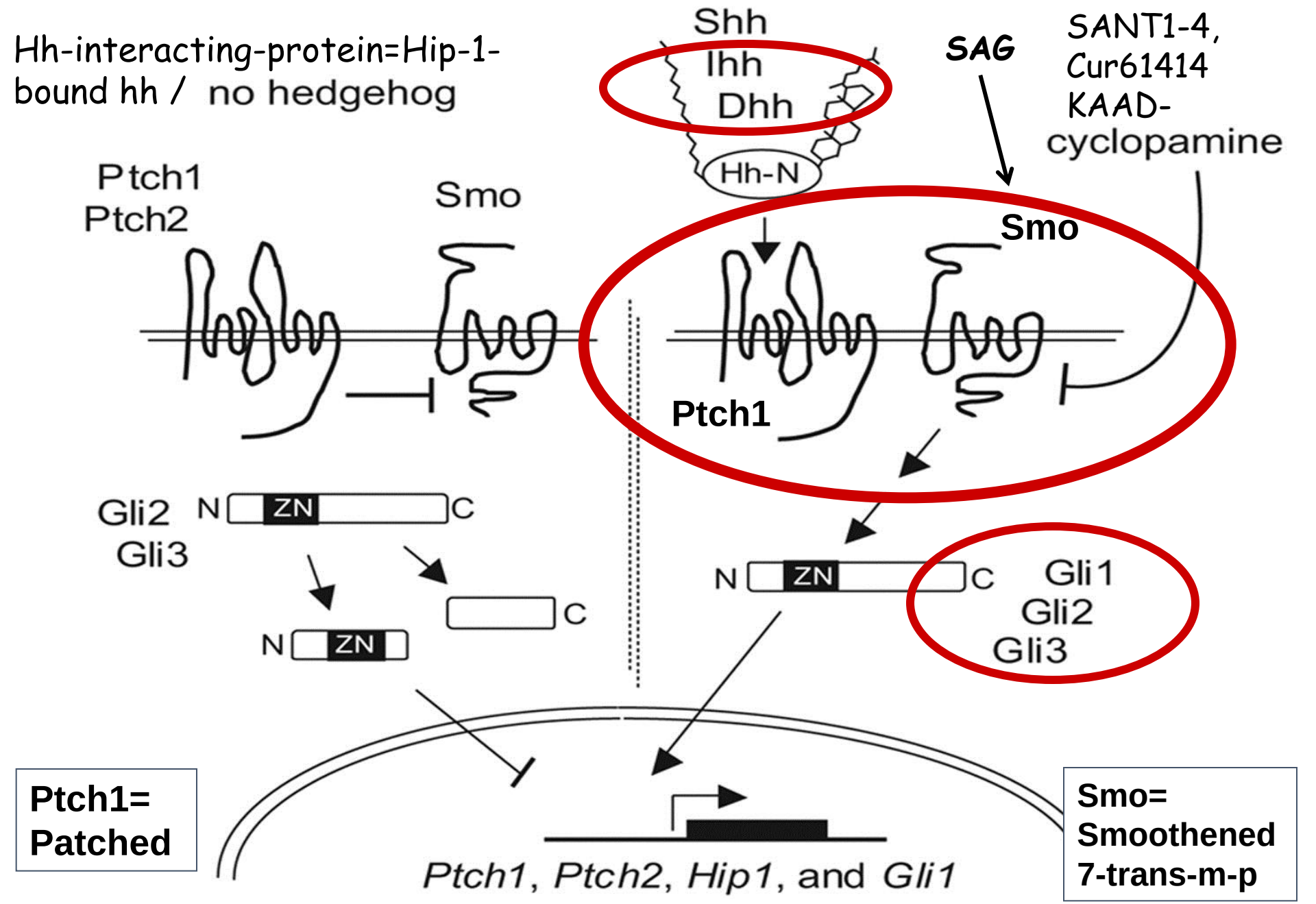


Modified from: Grado-Ahuir et al., 2011 (J. Anim. Sci. 89:1769-86)

Impact of hedgehog proteins on Thecal Cell Steroidogenesis

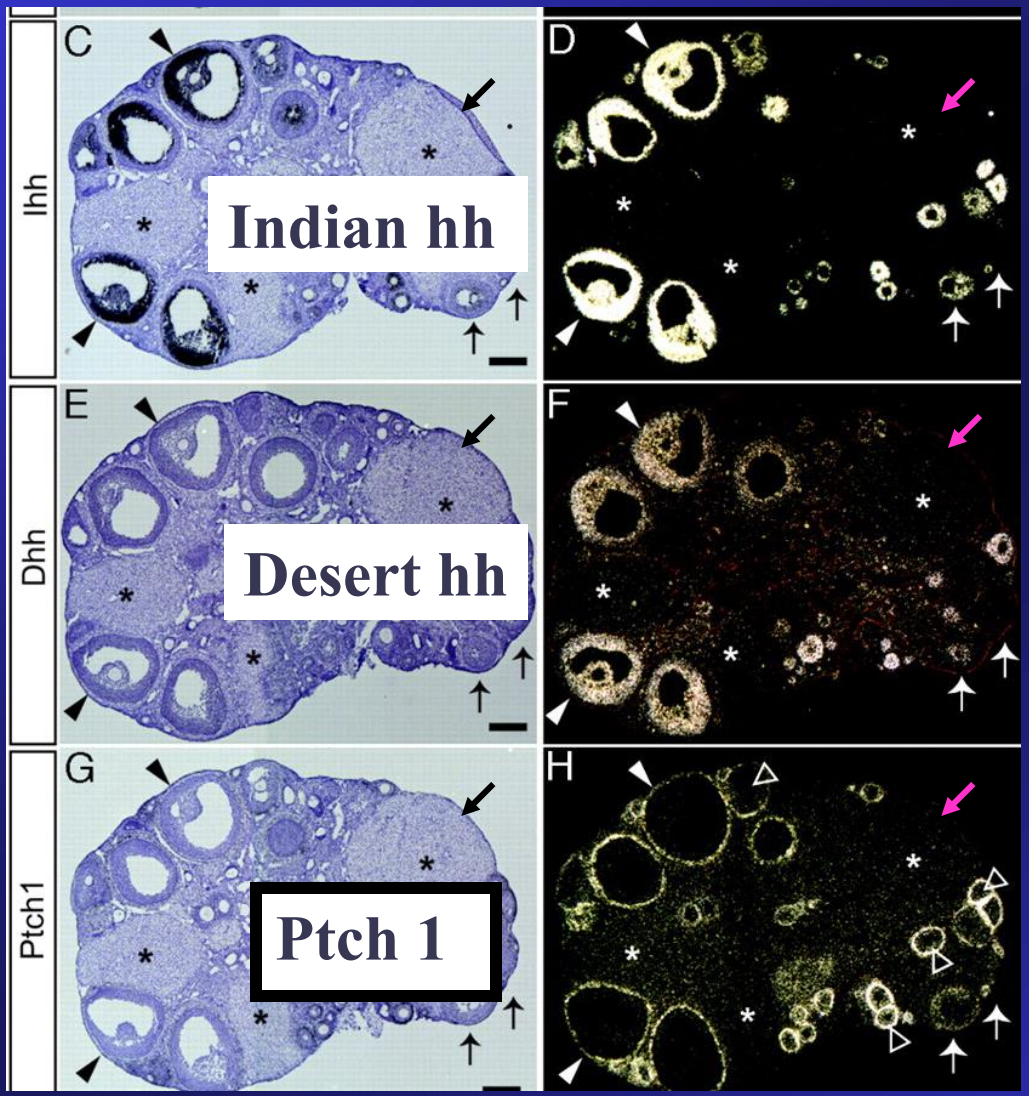


From: Cohen, 2003 (Amer. J. Med. Gen. 123A:5-28)



Modified from: Wijgerde et al., 2005 (Endocrinology 146:3558-66)

Cellular localization in postnatal mouse ovary



- **Ihh and Dhh** mRNAs located in GC of preantral and antral follicles (healthy).

- **Ptch1** mRNA mainly in adjacent theca cell compartment.

- None in CL.

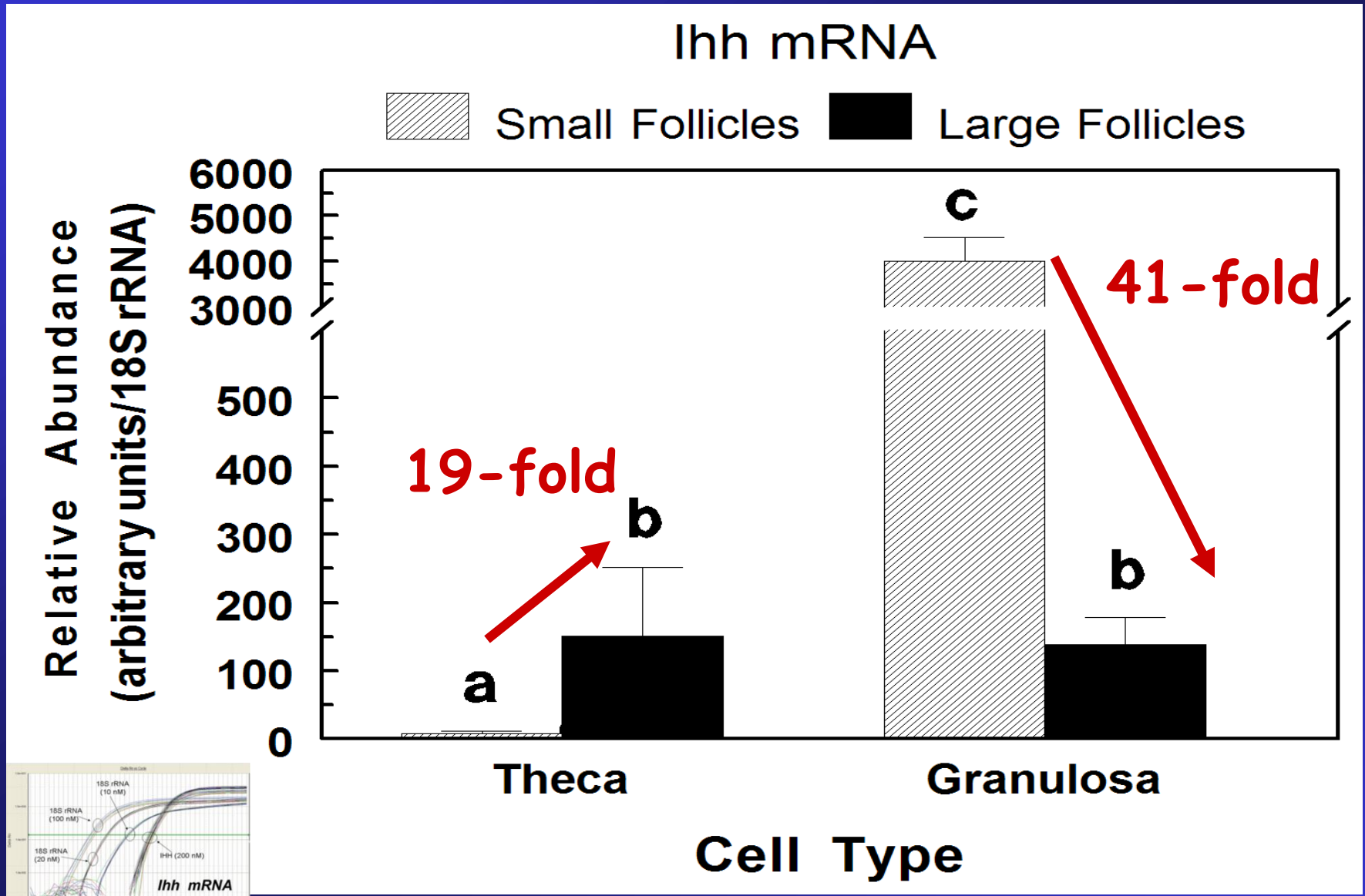
Large follicles

Atretic follicles

CL

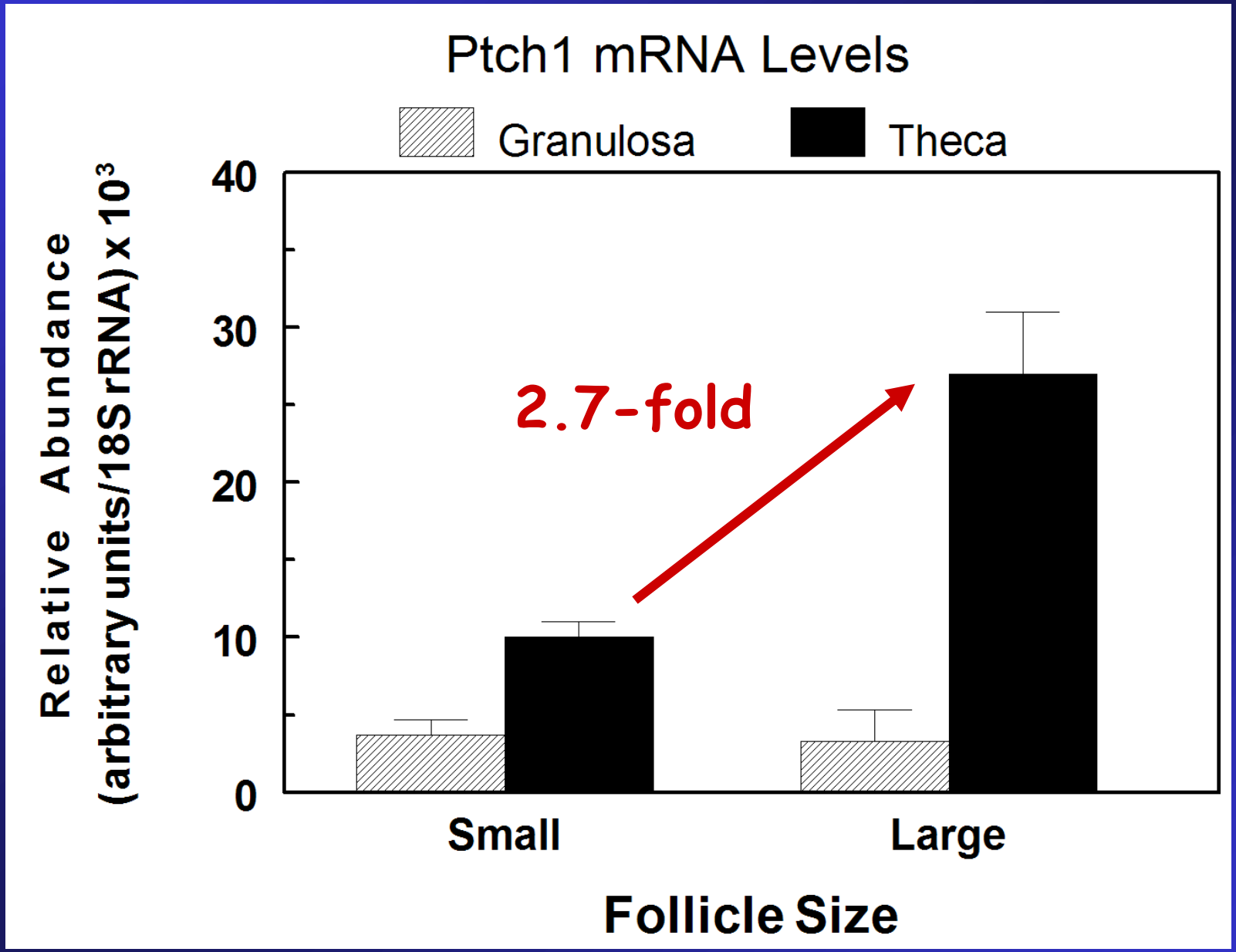
From: Wijgerde et al., 2005

Ihh mRNA in large vs. small bovine follicles



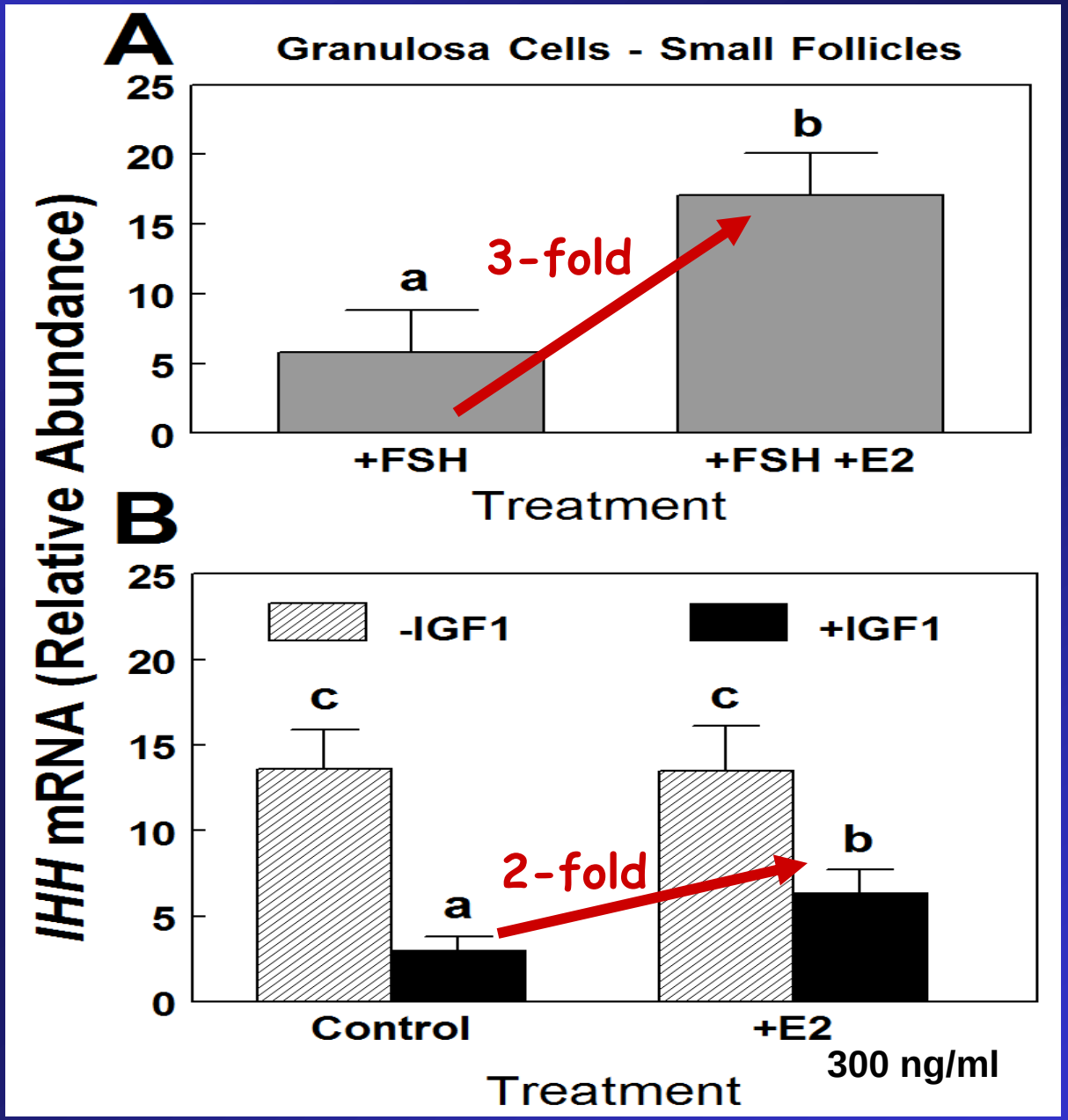
Redrawn from: Spicer, Sudo et al., 2009 (Reproduction 138:329)

Ptch1 mRNA is greater in large vs. small bovine follicles



Redrawn from: Spicer, Sudo et al., 2009 (Reproduction 138:329)

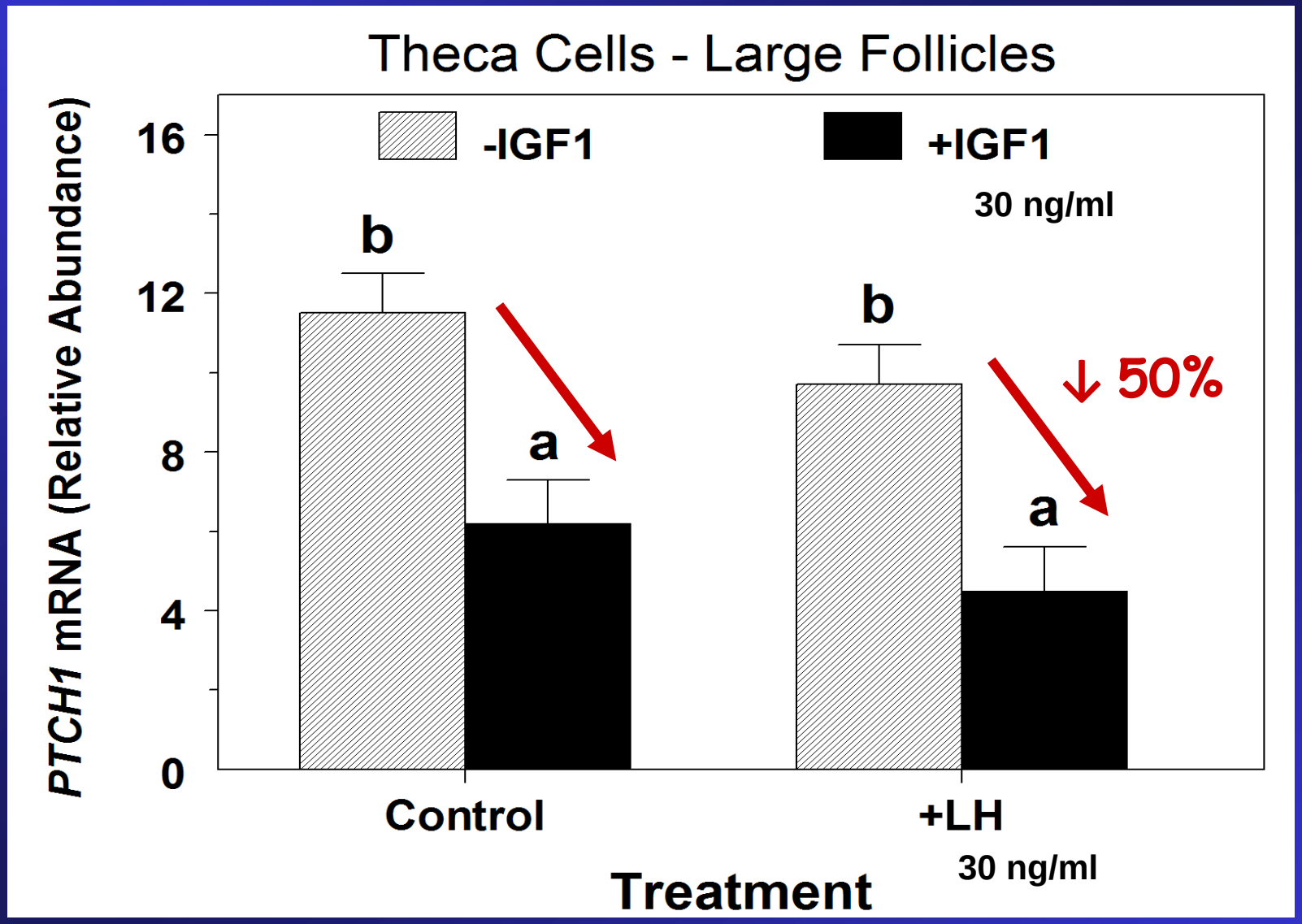
Estradiol stimulates & IGF1 inhibits *IHH* mRNA



From: Aad et al., 2012 (Biol Reprod 87:79)

FSH had no effect on *IHH* mRNA

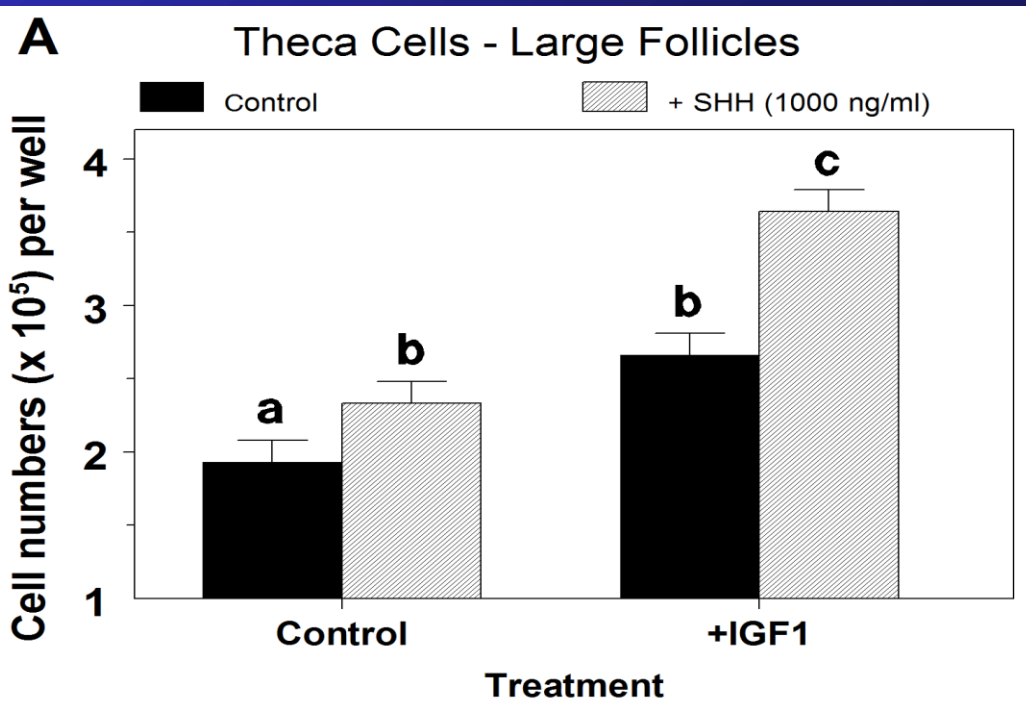
IGF1 inhibits theca cell *PTCH1*

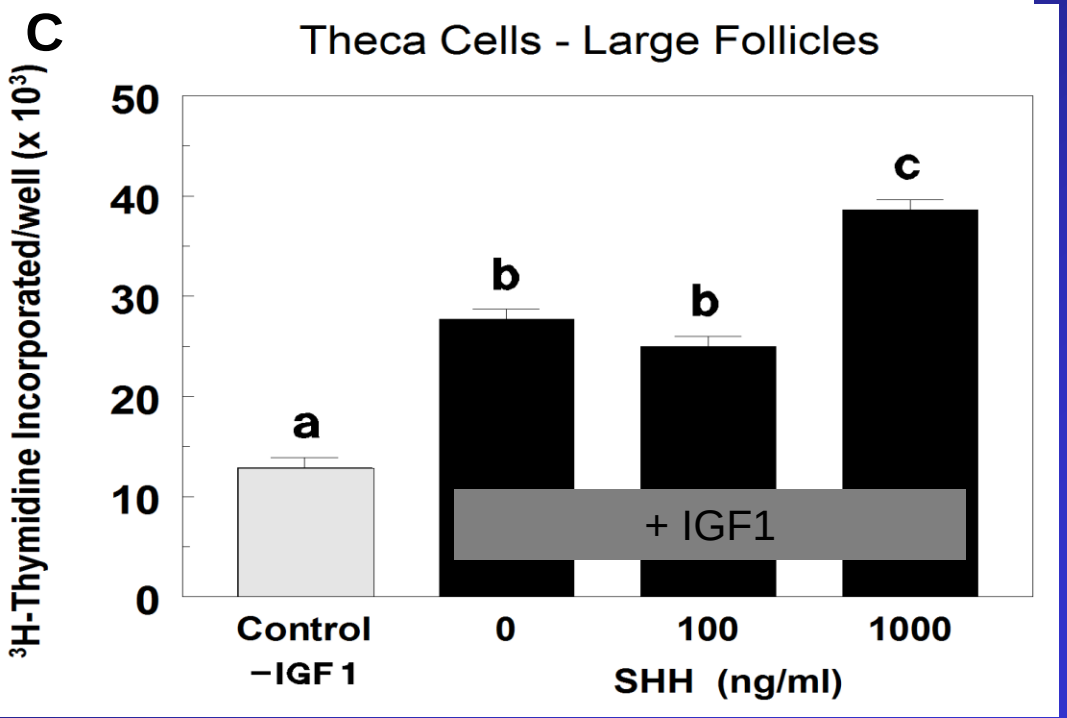


From: Aad et al., 2012 (Biol Reprod 87:79)

E2 increased *Ptch1* mRNA

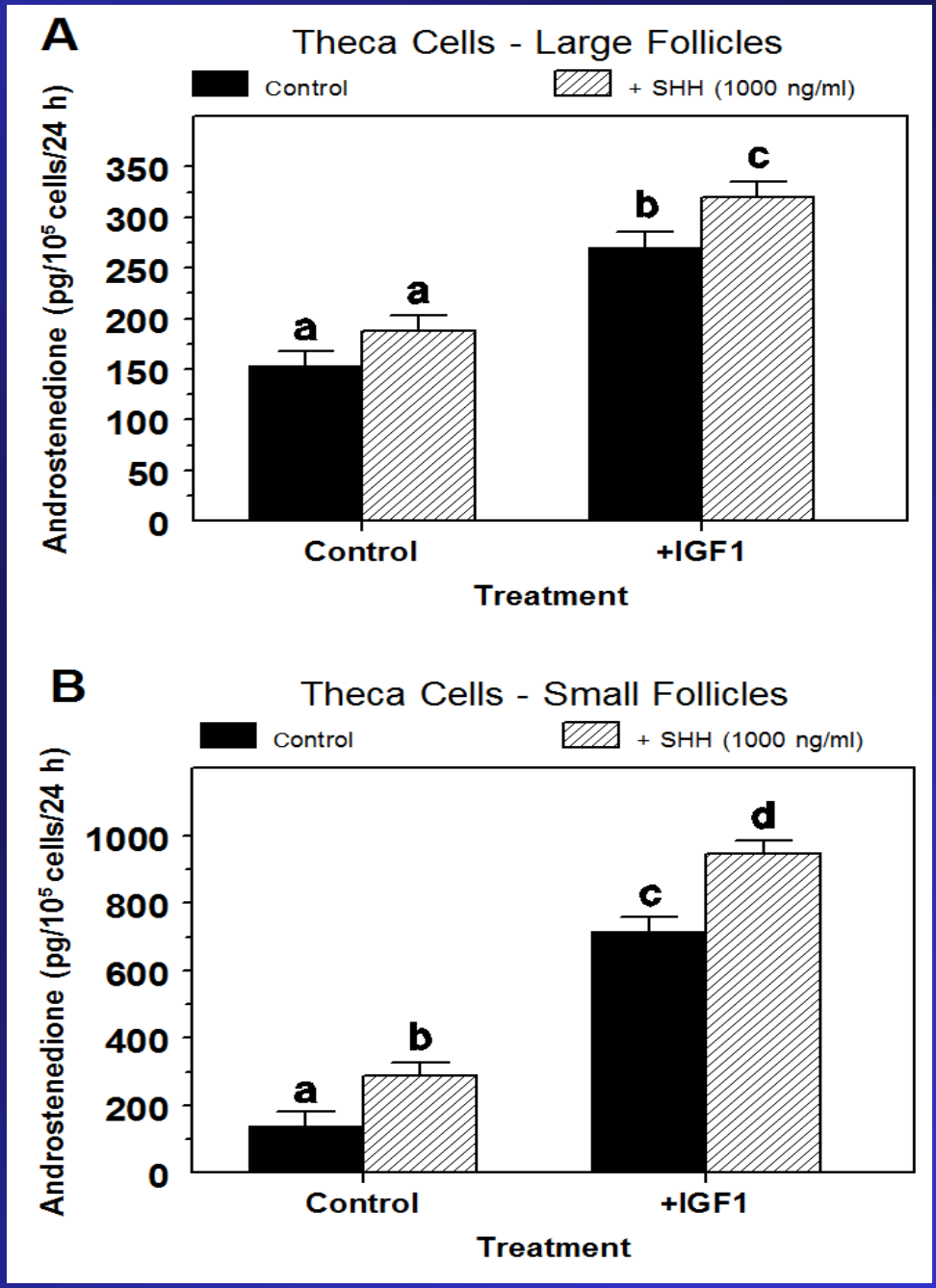
Hedgehog stimulates bovine theca cell proliferation





From: Spicer, Sudo et al., 2009 (Reproduction 138:329)

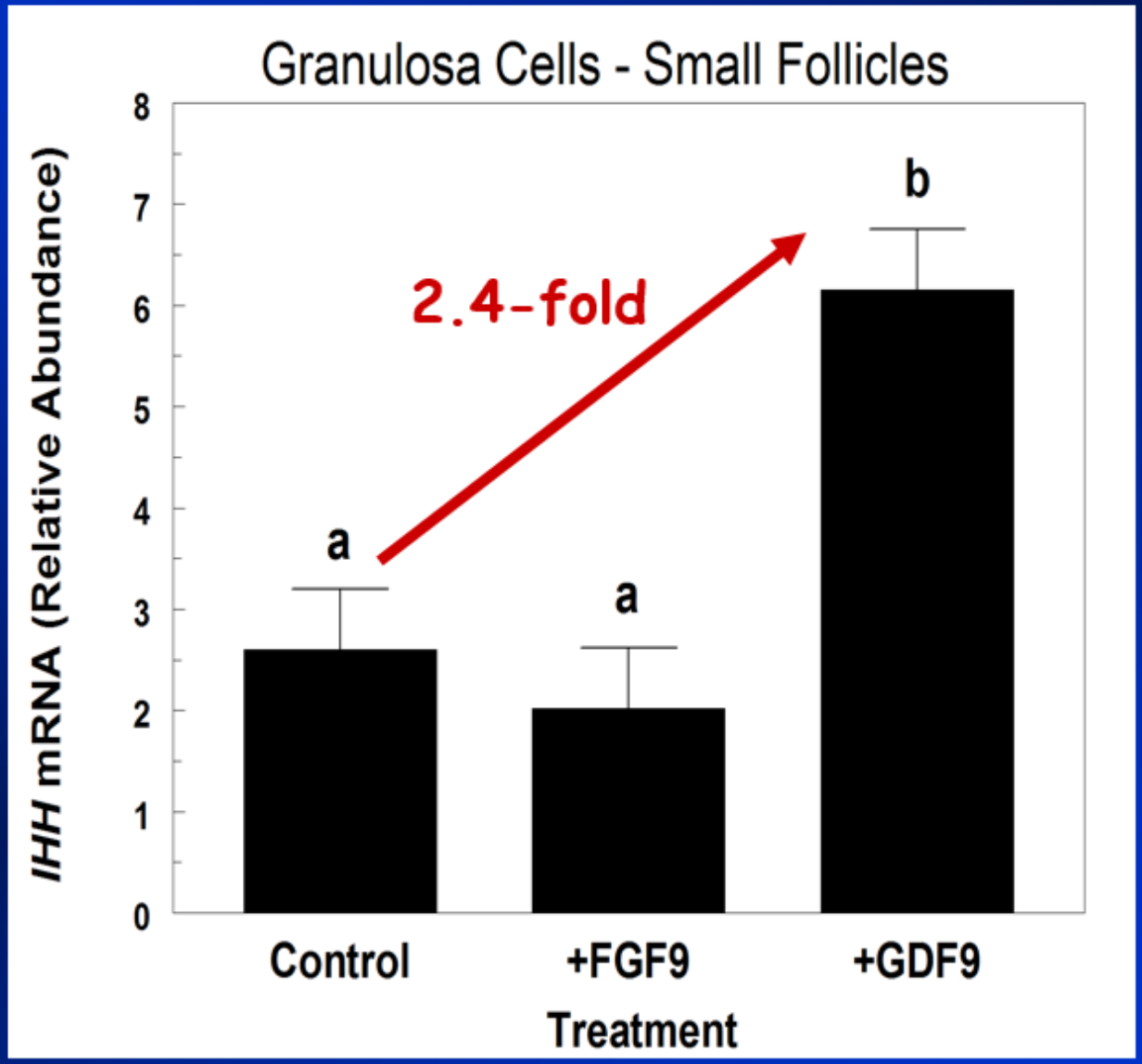
Hedgehog stimulates bovine theca cell androstenedione production



From: Spicer, Sudo et al., 2009 (Reproduction 138:329)

In cattle:

rm-GDF9 induces IHH mRNA in granulosa cells



From: Spicer & Schutz, 2015 (Unpublished)

Summary:

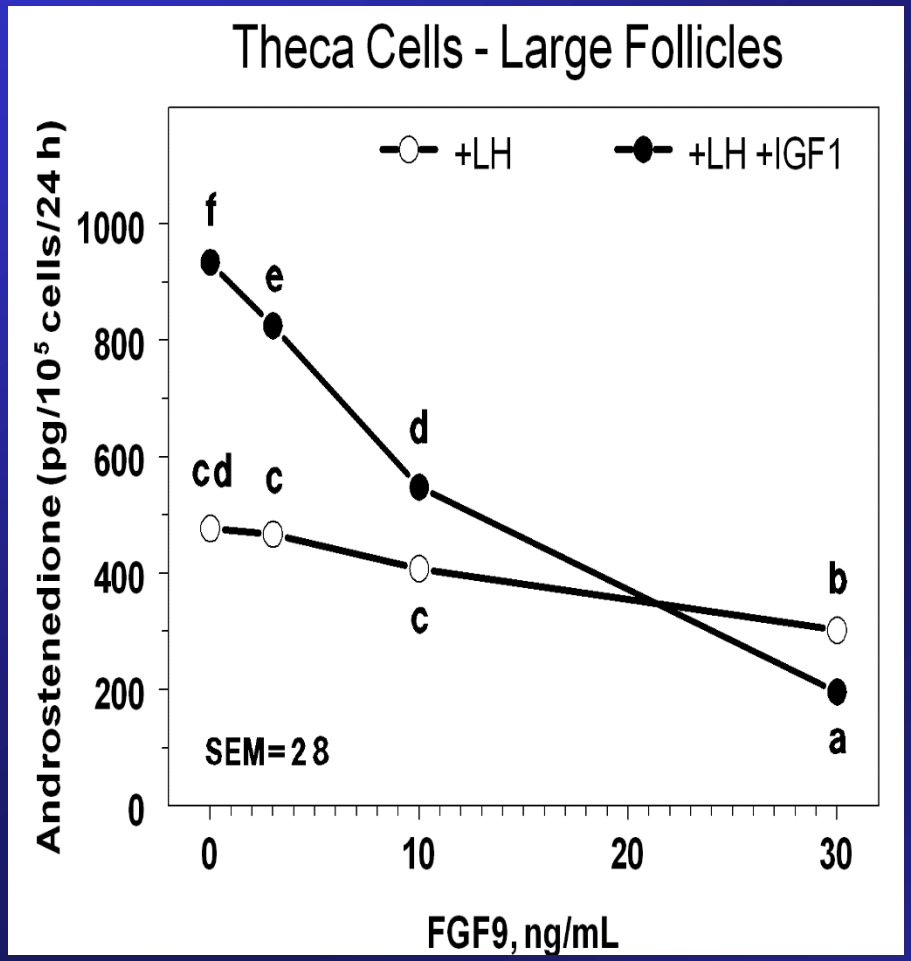
- **Hh proteins stimulate cell proliferation and stimulates gonadotropin-induced steroid production in bovine theca cells (Spicer et al., 2009).**
- **IGF1 inhibits Hh system in bovine granulosa & theca cells.**
- **GDF9 stimulates Hh system in bovine granulosa cells.**

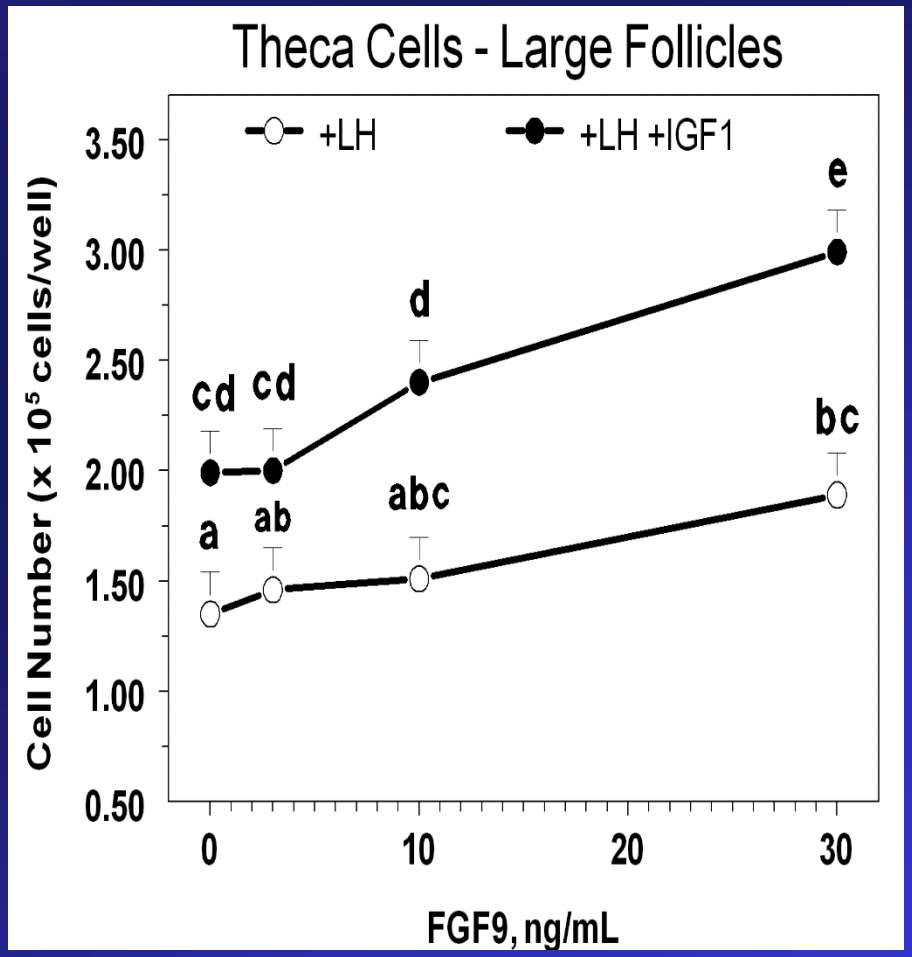
Impact of FGFs on Granulosa and Thecal Cell Steroidogenesis

FGF Family of Proteins

- **21 members of the FGF family signal through high affinity transmembrane tyrosine kinase receptors.**
- **There are 4 main FGF receptors (FGFR): FGFR1 through FGFR4.**
- **All receptors except FGFR4 have 2 to 3 isoforms (IIIa, IIIb, IIIc).**
- **FGF9 only binds to FGFR1IIIc, FGFR2IIIc, FGFR3IIIb, FGFR3IIIc, and FGFR4.**
- **Potential interactions with Heparin-related molecules.**

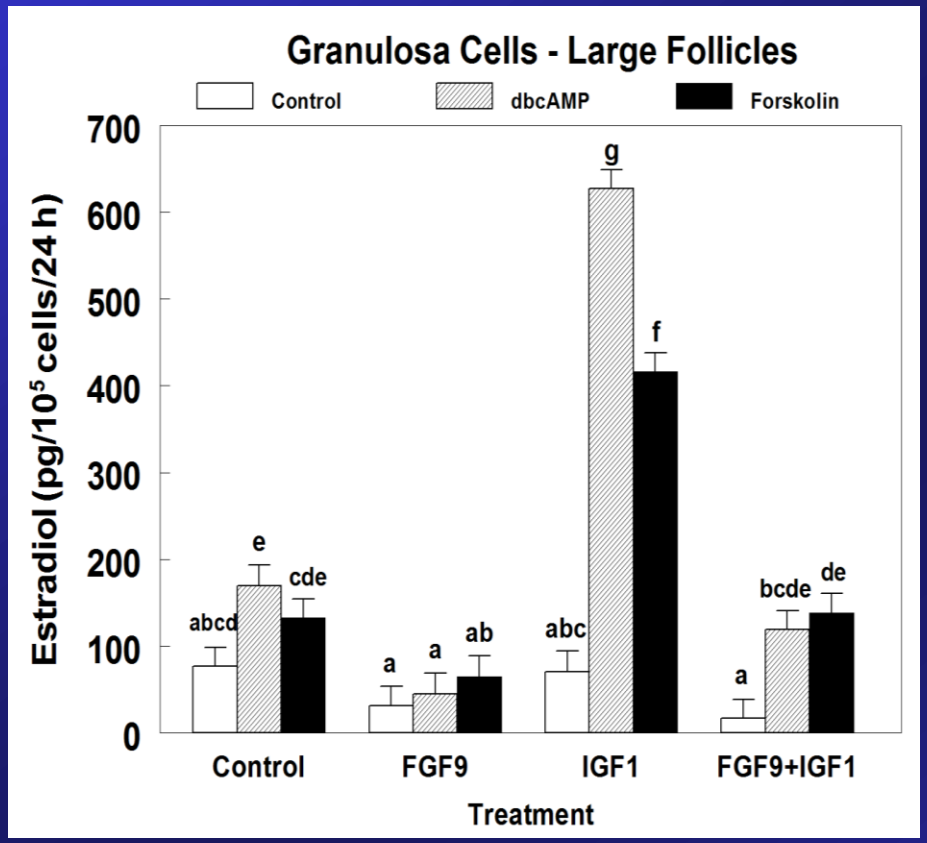
Steroid production is inhibited & proliferation is stimulated by FGF9 in bovine theca cells

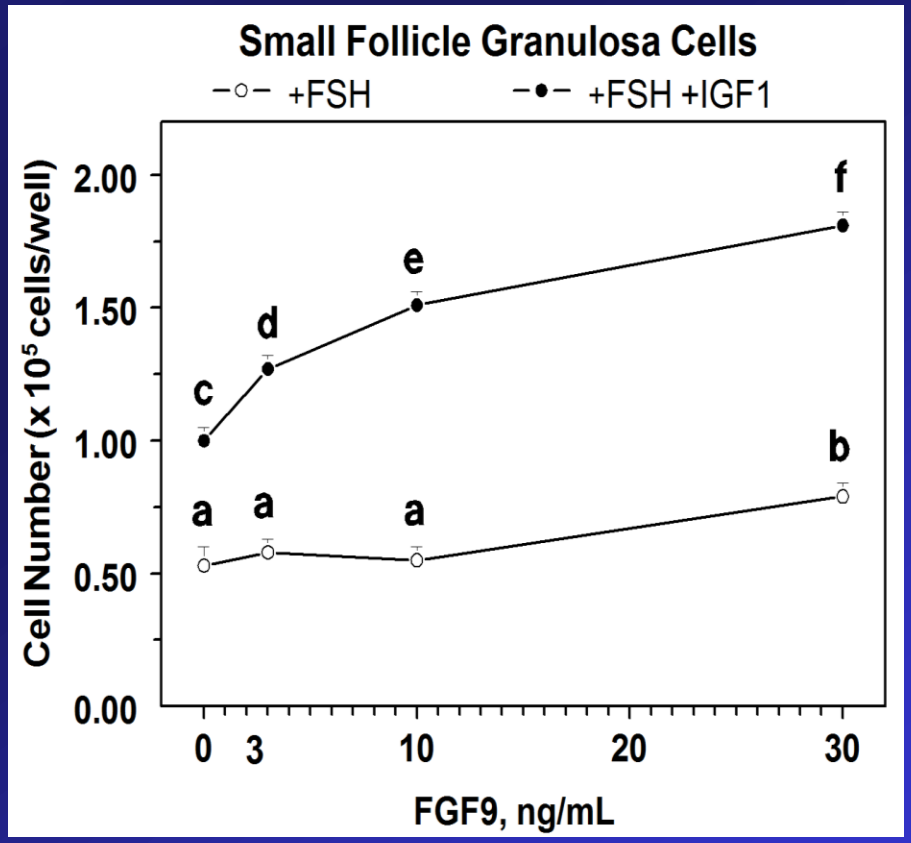




Schreiber et al., 2012 (J. Endocrinol. 215:167-75)

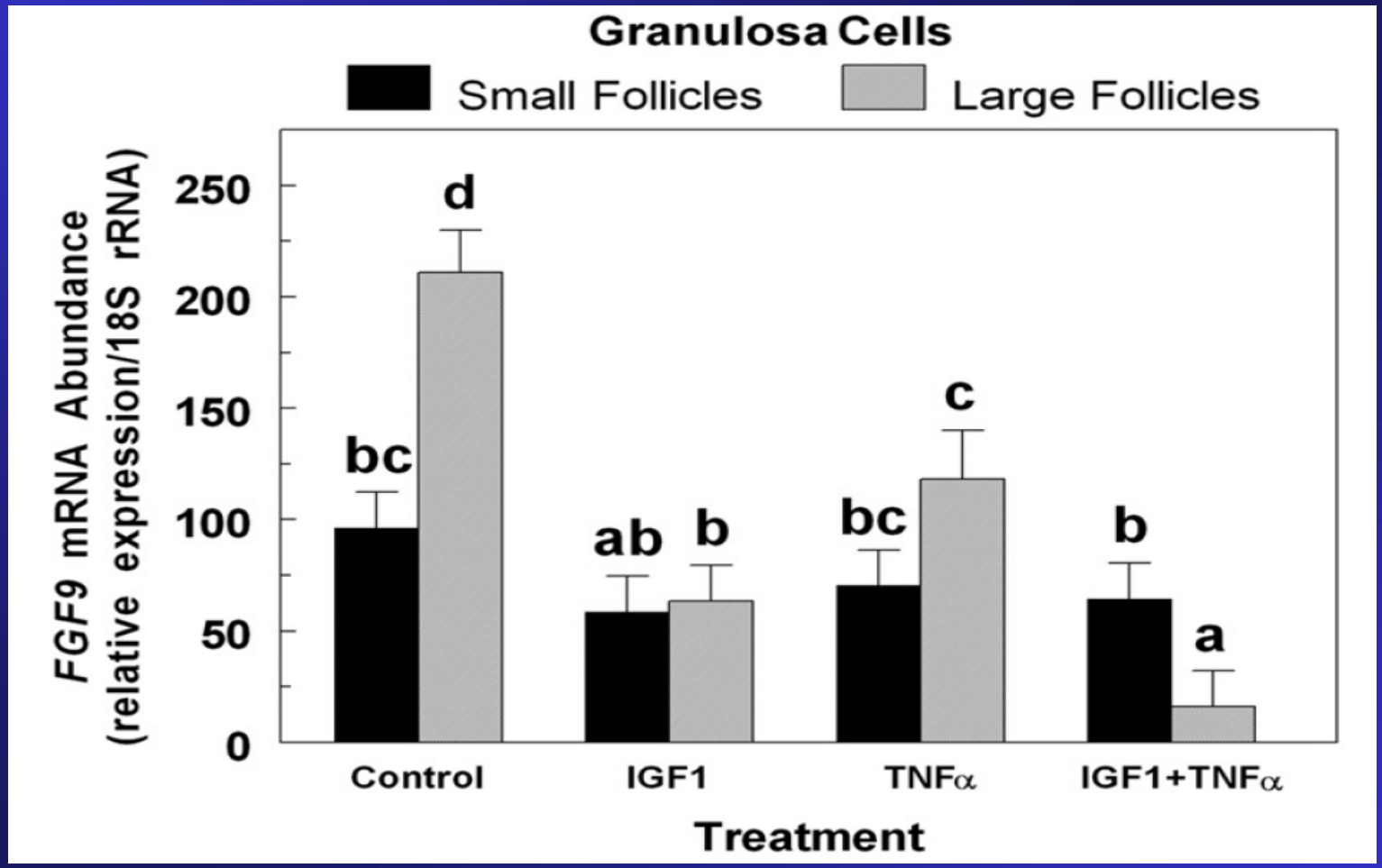
Steroid production is inhibited & proliferation is stimulated by FGF9 in bovine granulosa cells





Schreiber & Spicer, 2012 (Endocrinology 153:4491-501)

FGF9 mRNA is inhibited by IGF1 & TNFα in bovine granulosa cells

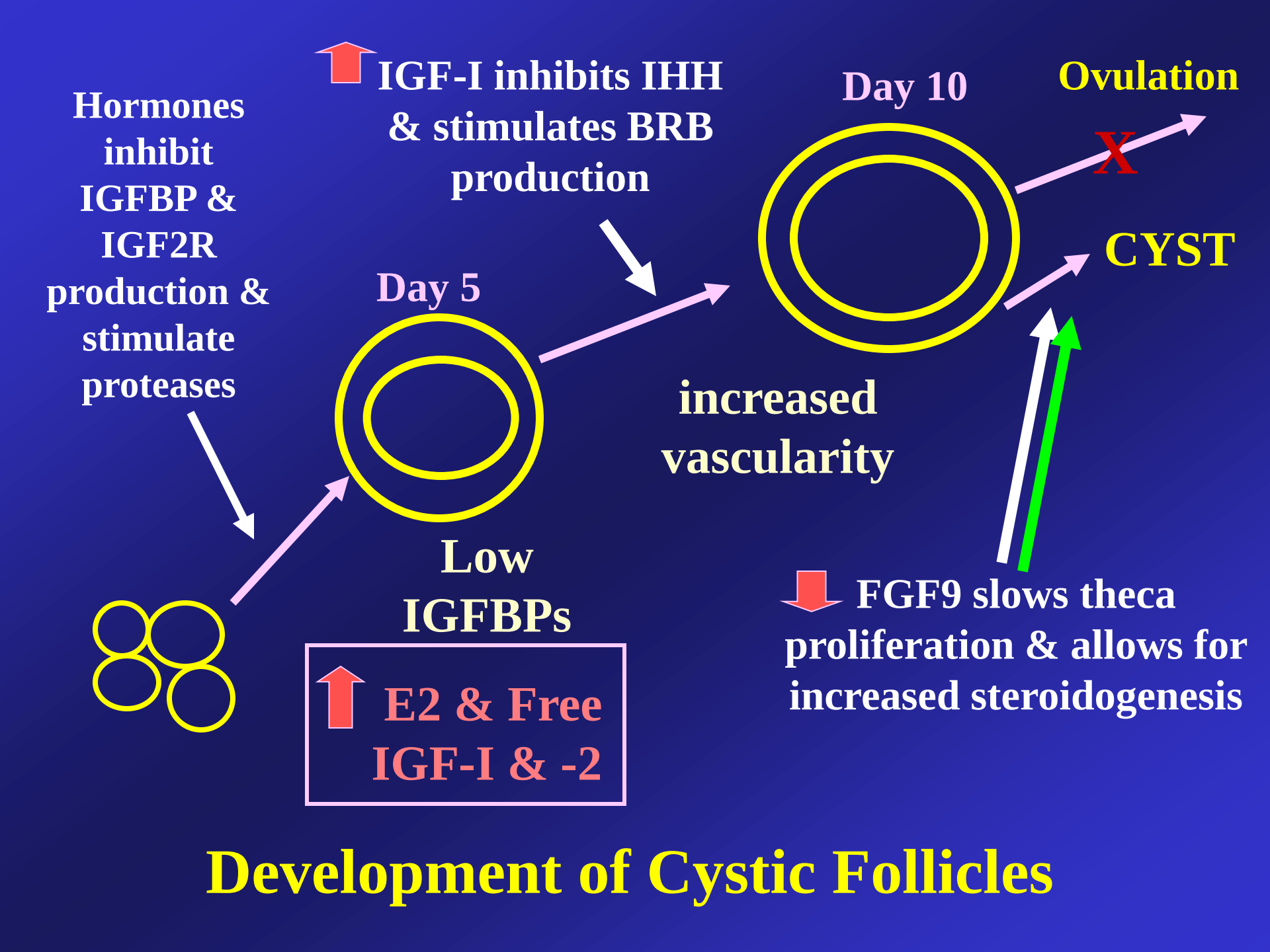


Schreiber & Spicer, 2012 (Endocrinology 153:4491-501)

Summary:

- **FGF9 stimulates cell proliferation and inhibits gonadotropin-induced steroid production in bovine granulosa & theca cells (Schreiber & Spicer, 2012; Schreiber et al., 2012).**

- **IGF1 & TNFα inhibits FGF9 mRNA in bovine granulosa cells.**



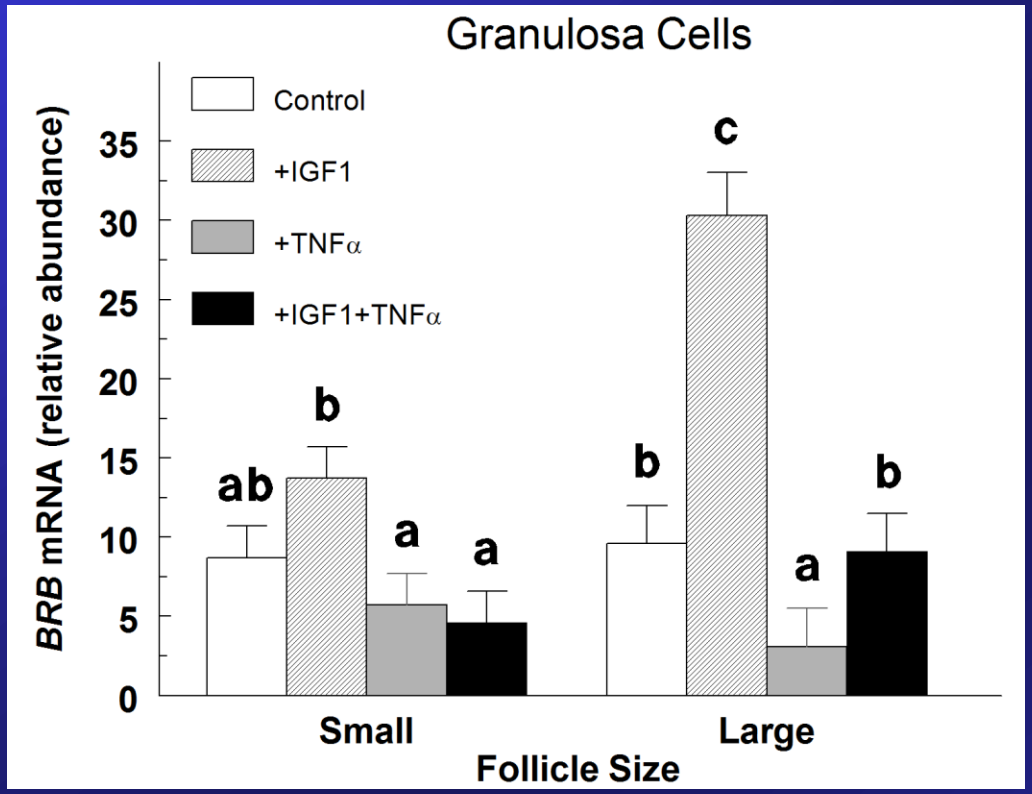
Development of Cystic Follicles

Conclusions:

- **Can microarray studies help identify new factors? YES**

- **GENES identified point toward IGF-1 & growth factor regulation of mitogenic & angiogenic factors.**

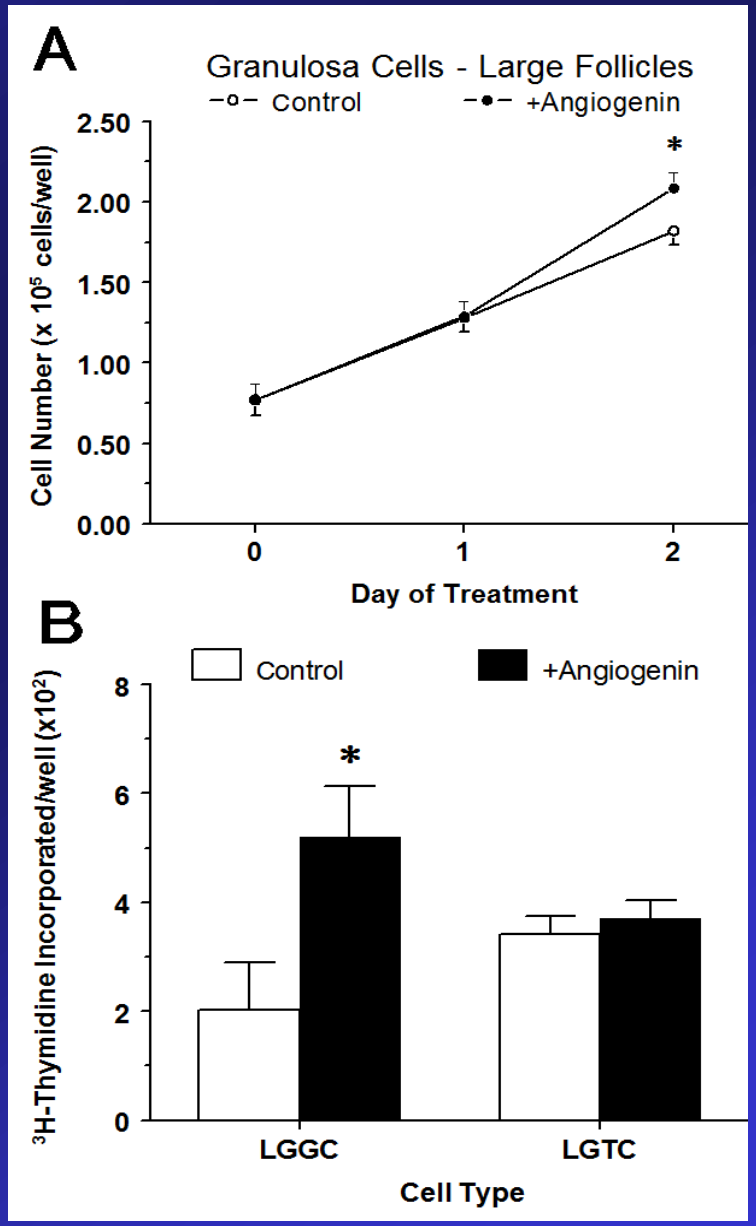
In Bovine Granulosa Cells

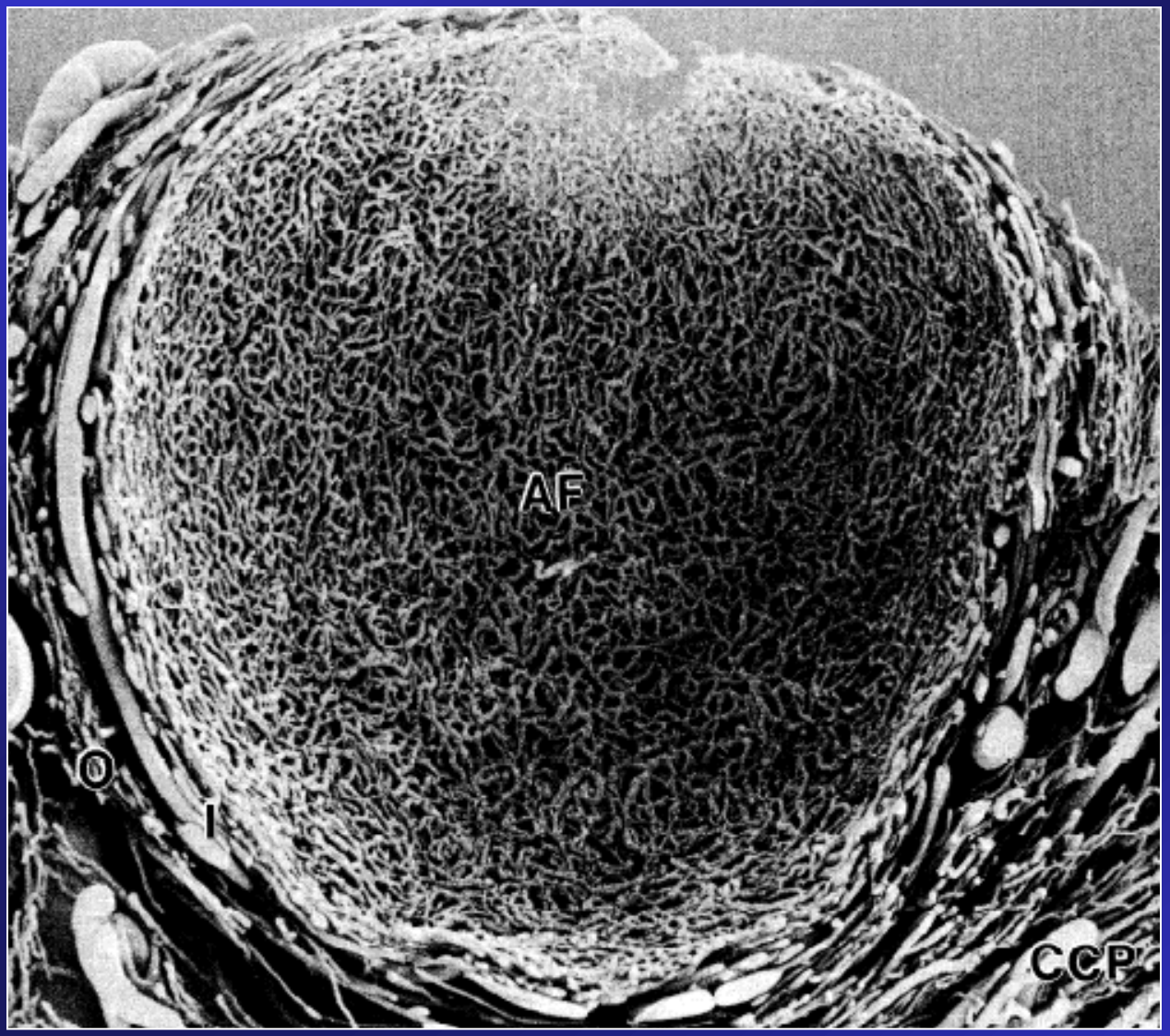


Bovine Brain Ribonuclease is induced by IGF1

Spicer, unpublished

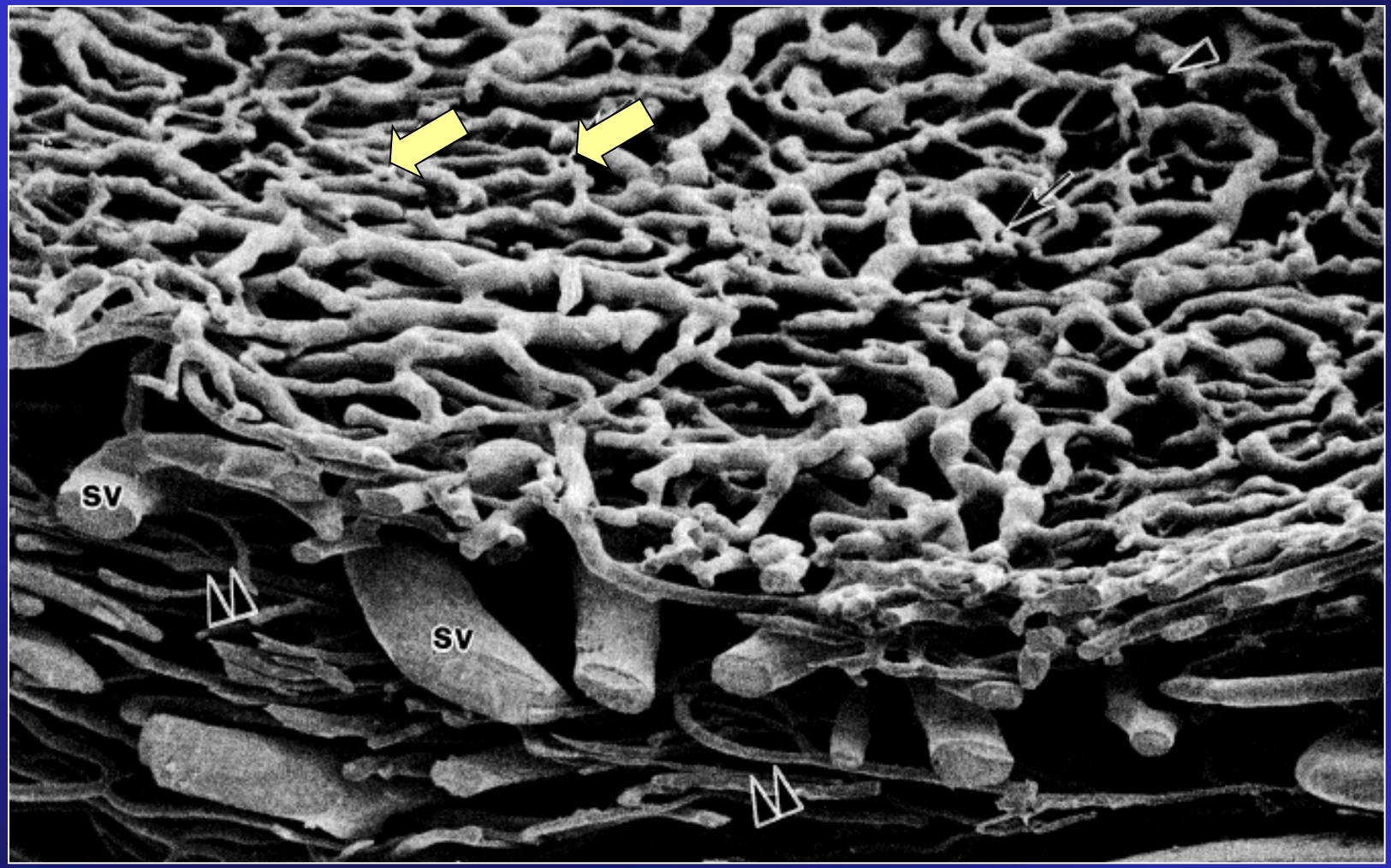
Angiogenin stimulates proliferation





Vascular Cast of 5 mm Bovine Follicle

Yamada et al., 1995 (Arch. Histol. Jap. 58:567)



Vascular Cast of Bovine Antral Follicle

Yamada et al., 1995 (Arch. Histol. Jap. 58:567)

Conclusions:

- **IGFs & IHH may regulate mitogenic & angiogenic factors produced by the follicle, including cell proliferation while stimulating FSH/LH-induced differentiation.**

- **GDF9, BMP4 & FGF9 may induce mitosis & ihibit steroidogenesis of granulosa & theca cells.**

- **Ribonuclease A proteins (i.e., BRB & ANG) may be physiologically relevant and hormonally regulated in granulosa cells.**

Future Directions:

Will alteration in these systems:

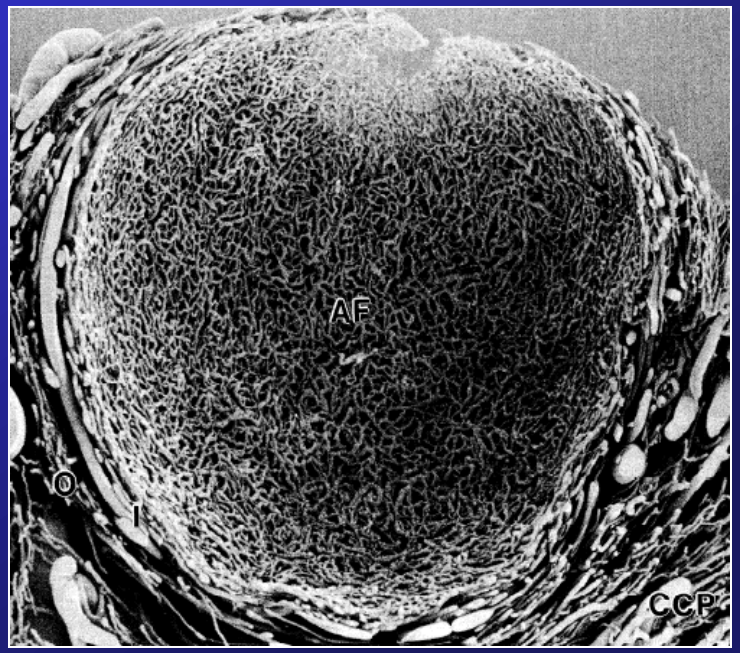
1. ***Reduce the number of follicles that undergo atresia?***
2. ***Improve a female's response to superovulation/ synchronization?***
3. ***Prevent development of cystic ovaries?***

Oklahoma State University

- A great place to do research

Dziekuje

Cyst Hypothesis - Cow:



Angiogenesis

Theca cell

A4

IHH FGF9

Proliferation

?

IGF1Rc

IGF1 & 2
(↑ Free)

Angiogenin

TNFα

Granulosa cell