Inhibin-B secretion and FSH isoform distribution may play an integral part of follicular selection in the natural menstrual cycle

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Take home message

- Inhibin-B secretion is maximal during the mid-follicular phase stimulated predominantly by acidic FSH isoforms
- Inhibin-B exerts an important endorine function by down regulating FSH secretion during the mid-follicular phase
- Inhibin-B secures a high output of androgens from the theca cells
- LH is mainly driving follicular development in the second half of the follicular phase

The ovarian follicle
The functional unit with endocrine functions

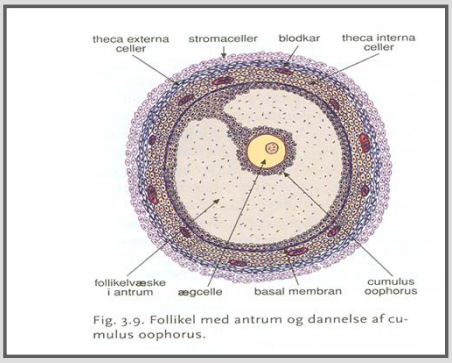
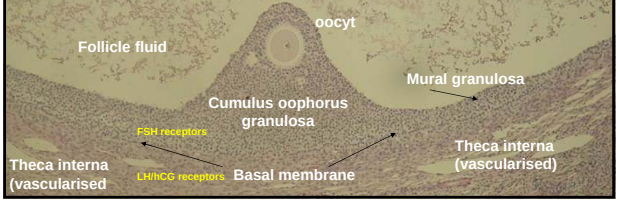


Fig. 3.9. Follikel med antrum og dannelse af cumulus oophorus.

Human antral follicle 5mm in diameter

Functions of the follicle:

1. To keep and protect female gametes
2. To establish an intra-follicular milieu, which supports the growth and maturation of the oocyte
3. To corporate with the hypothalamus/pituitary – gonadal system to secure feed-back and development of only one follicle
4. Via its endocrinological activity to prepare extra gonadal organs for pregnancy
5. To release the fully mature oocyte at ovulation facilitating fertilization
6. To convert its endocrinological activity after ovulation to support implantation and early pregnancy

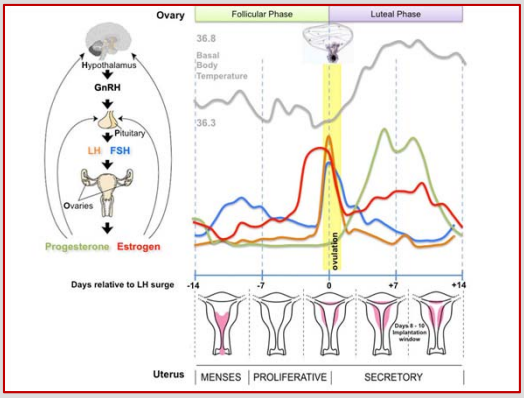


Antral follicle: vacularity of theca and granulosa cells

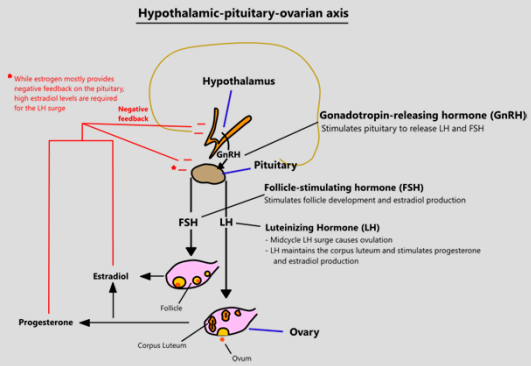
Source: AJ Zeleznik

Human adult ovary

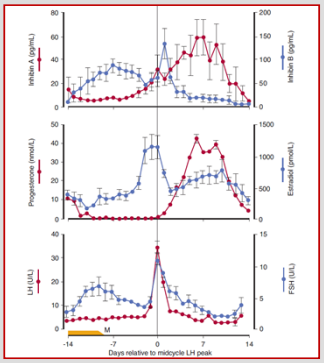
The hypothalamus-pituitary-gonadal axis (HPG)



The textbook view:



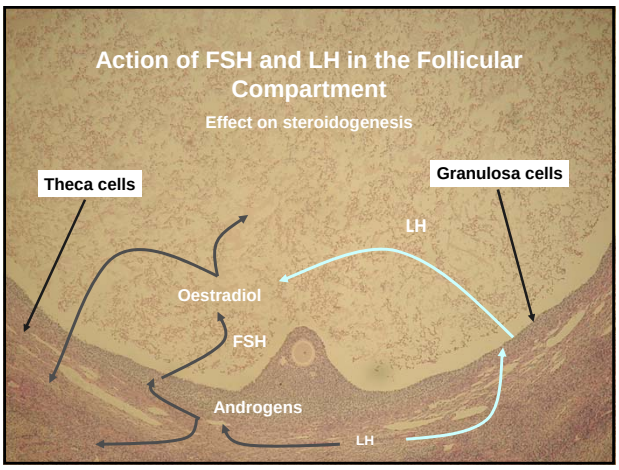
Natural Hormonal Cycle in Women



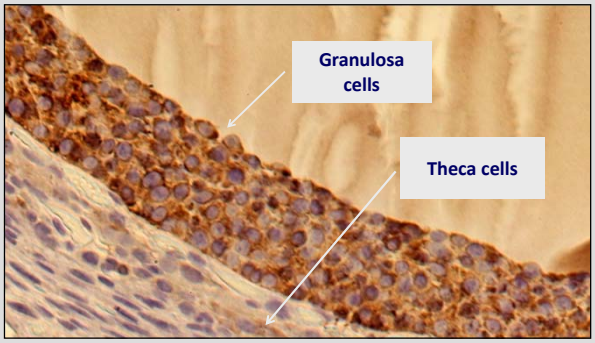
Groome N *et al.*, *JCEM*, 1996;**81**:1401

Action of FSH and LH in the Follicular Compartment

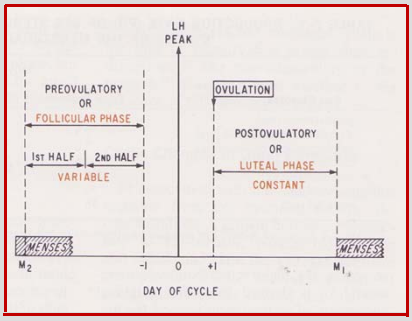
Effect on steroidogenesis



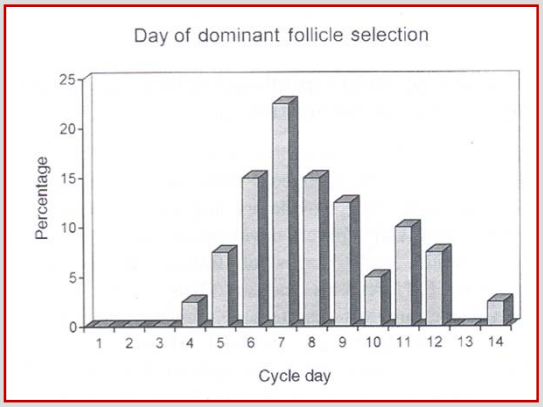
Aromatase (CYP19) expression in human granulosa cells



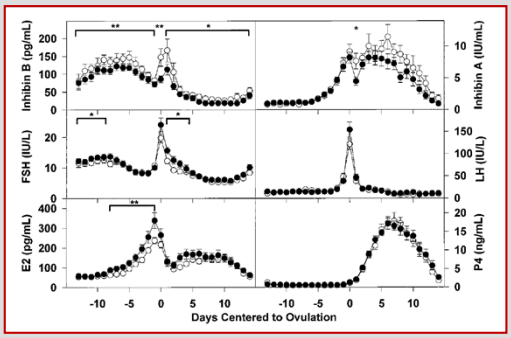
The human menstrual cycle



S. Yen & Jaffe, 1979



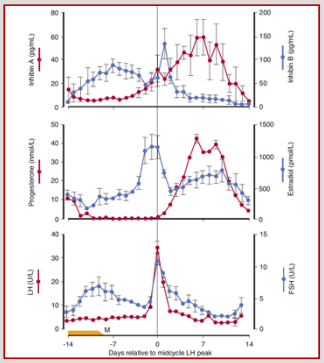
Female reproductive aging is marked by decreased secretion of inhibin-B



Women 20—34 years Women 35—46 years

Welt CK *et al.*, *JCEM*, 1999;**84**:105

Natural Hormonal Cycle in Women

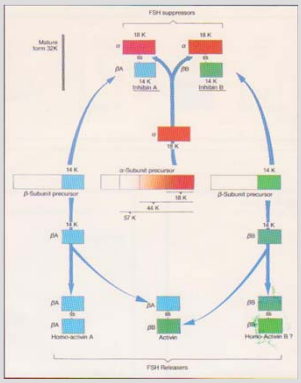


Groome N *et al.*, *JCEM*, 1996;**81**:1401

Women with granulosa cell tumors

- Often hypersecrete inhibin-B or AMH
- Concentrations of around 150 pg/ml with cause anovulation
- Once the tumor is removed levels of inhibin-B normalises and ovulatory cycles continues

Molecular structure of inhibin's and activin's

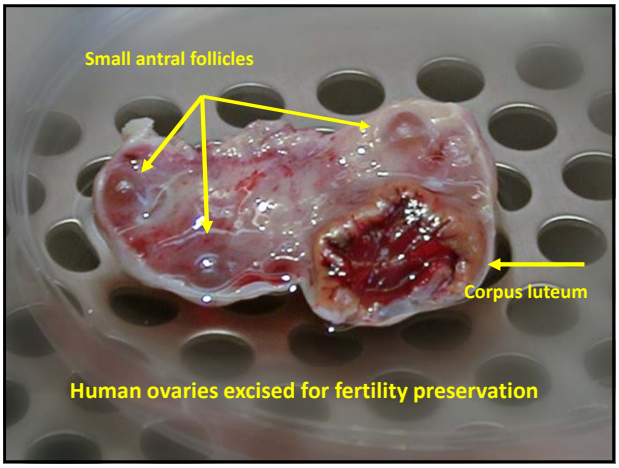


OK
Inhibin-B rather than oestradiol is the main down-regulator ff FSH secretion in the early follicular phase

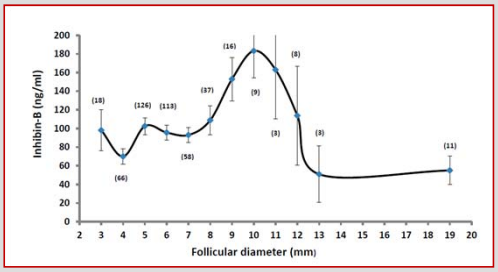
I hope you are still onboard

Inhibin-B is only produced by granulosa cells

Is the peak in circulation mirrored by a peak in follicles from the natural menstrual cycle?



Concentrations of inhibin-B in follicular fluid in relation to the follicular diameter: natural menstrual cycles

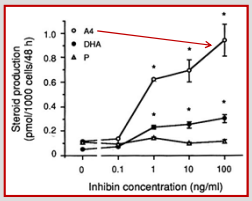


One effect of inhibin-B is to down-regulate FSH secretion in the mid-follicular phase of the natural menstrual cycle

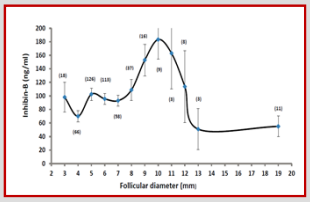
Yding Andersen *et al., Hum. Reprod.* 2010;**25**:1282

Inhibin-B drives thecal androgen synthesis in synergy with LH and intrafollicular levels peak at cycle day 7 – 9

Theca cells in culture



Natural cycle



One other function of inhibin-B is to act on theca cells in paracrine manner

Hillier *et al., Mol.Cell. Endocrinol.*, 1991;**75**;R1

Yding Andersen *et al., Hum. Reprod.* 2010;**25**:1282

Does inhibin-B augment androgen production *in vivo*?

Follicular fluid concentrations of testosterone in human small antral follicles in relation to concentrations of inhibin-B and steroids

Testosterone concentration (nmol/L) (Mean ± SEM)					
	< 70	70 – 130	130 - 280	>280	ANOVA
Number	22	24	29	25	
Inhibin-B (ng/ml)	63 ± 11	50 ± 11	105 ± 18	132 ± 25	P < 0.009
Androstenedione (nmol/L)	120 ± 11	346 ± 31	701 ± 51	1007 ± 114	P <0.001
Progesterone (nmol/L)	176 ± 36	302 ± 63	428 ± 62	359 ± 59	P <0.03
Oestradiol	36 ± 19	49 ± 21	156 ± 45	209 ± 82	P >0.10

Yding Andersen C *et al., JCEM* 2008;**93**:2344

Follicular fluid concentrations of testosterone in human small antral follicles in relation to mRNA expression of *FSHR, LHR, CYP19* and *AMHR-2* genes in the corresponding granulosa cells

Testosterone concentration (nmol/L) (Mean ± SEM)					
	< 145	146 – 210	211 - 350	>350	Spearman
Number	15	13	13	13	
FSH-R (x1000)	52 ± 17	49 ± 16	122 ± 35	185 ± 51	P < 0.003
LH-R (x1000)	1,0 ± 0,3	0,7 ± 0,2	1,2 ± 0,6	0,8 ± 0,3	P >0.10
AMHR-2 (x1000)	10 ± 2	25 ± 10	17 ± 4	20 ± 5	P >0.10
CYP-19 (x1000)	38 ± 16	46 ± 22	36 ± 13	19 ± 9	P >0.10

Follicular fluid concentrations of Androstenedione shows a similar significant association to FSHR mRNA expression

Nielsen ME et al., *Mol Hum Reprod.* 2011;**17**:63

Gene Expression in Human Granulosa Cells from Follicles 3 – 9 mM

Androgen Receptor mRNA expression (x1000) (Mean ± SEM)					
	< 20	20 – 45	> 45 –130	> 130	Spearman
Number	11	16	14	14	
FSH-R (x1000)	18 ± 8	56 ± 13	133 ± 31	181 ± 49	P <0.0001
LH-R (x1000)	0,63 ±0,27	0,53 ±0,19	0,87 ±0,22	1,76 ±0,69	P <0.02
AMHR-2 (x1000)	4,6 ± 1,4	21,2 ± 7,8	16,2 ±3,8	23,2 ± 3,1	P <0.0008
CYP-19 (x1000)	38 ± 21	33 ± 16	19 ± 7	48 ± 15	P >0.10

No significant relation to Follicular Fluid hormone parameters AMH, Inhibin-B, Oestradiol, Progesterone, Androstenedione and Testosterone

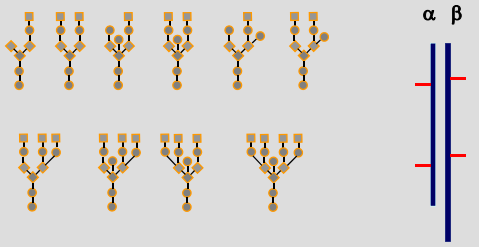
Nielsen ME et al., *Mol Hum Reprod.* 2011;**17**:63

Why does inhibin-B peak in the mid-follicular phase of the menstural cycle

- Inhibin-B is produced by the granulosa cells
- FSH stimulate inhibin-B secretion

FSH is not just FSH

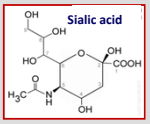
differencies in glycosylation

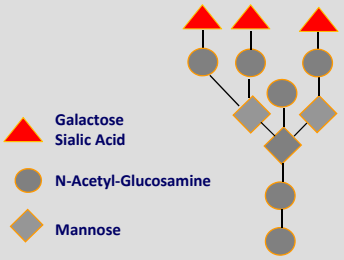


More than 35 different oligosaccharides have been identified

Glycans – sugarresidues – oligosaccharides – carbohydrates – glycosylation

FSH is not just FSH



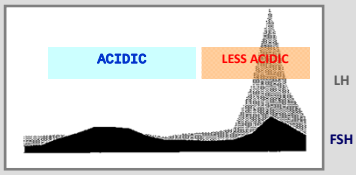


In vivo characteristics of FSH isoforms

- The circulatory half-life of acidic isoforms is longer than of less acidic (De Leeuw et al., *Mol.Hum.Reprod*, 1996, 2, 361)
- The collective *in vivo* activity is higher of acidic isoforms as compared to less acidic
- The presence of more sialic acid residues on the acidic isoforms shields galactose residues and prevents hepatic asialoglycoprotein receptors from removing proteins from circulation

In vivo characteristics of FSH isoforms

- Acidic isoforms predominate in the early follicular phase whereas less acidic isoforms are more common as ovulation approaches. (Anobile et al., *Mol. Hum. Reprod*, 1998, **4**, 630)

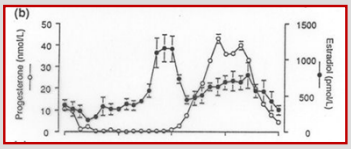


In vivo characteristics of FSH isoforms

The follicle itself by secreting E_2 regulates the pituitary FSH isoform release

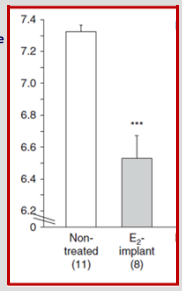
- Rising E_2 levels cause a shift towards less acidic isoforms (Padmanbhan et al., *JCEM*, 1988, **67**, 465)
- Glycosyltransfereses, (e.g. 2,3 α-sialyltransferase) which are enzymens that catalyse the incorporation of sialic acid residues into FSH within the pituitary become down-regulated by E_2

Oestradiol levels during the natural menstrual cycle



Effect of administrating oestradiol to postmenopausal women on sialic acid content of FSH

Number of sialic acid residues per FSH molecule



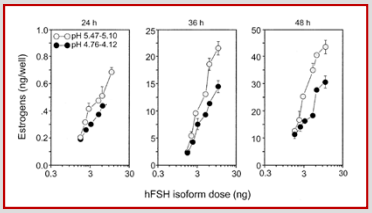
Wide L *et al., Upsala J. Med. Sci.* 2010;**115**:97

FSH-isoforms provoke a diverse biological response *in vitro*

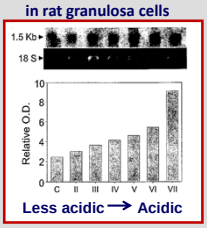
Less acidic: Enhanced E_2 production by granulosa cells obtained before and after the midcycle surge of gonadotropins
Augmented cAMP production by granulosa cells

Acidic: Augmented Inhibin production by granulosa cells

Production of oestradiol of mouse follicles in culture

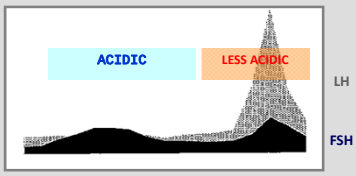


α-inhibin subunit mRNA in rat granulosa cells

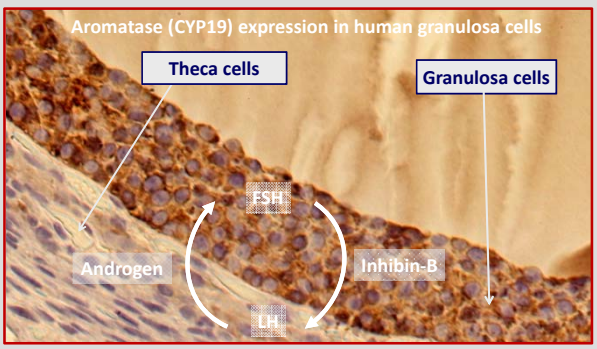


FSH action in first half of the follicular phase of the natural menstural cycle

FSH and predominantly acidic FSH isoforms stimulate graunulsoa cells synthesis of Inhibin-B which peaks around follicular selection and when increase in E_2 starts to take off resulting in a shift to less acidic isoforms begins



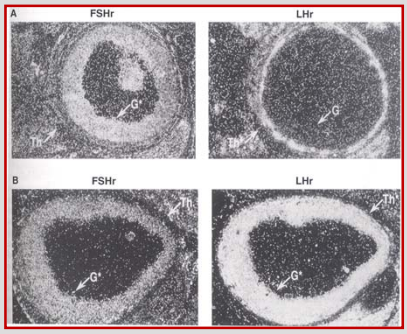
Acidic FSH isoforms – in particular – derives via inhibin-B thecal cell androgen production around follicle selection



Expression of FSHR and LHR in an antral follicle and in a pre-ovulatory rat follicle adjacent sections

Antral follicle

Preovulatory follicle

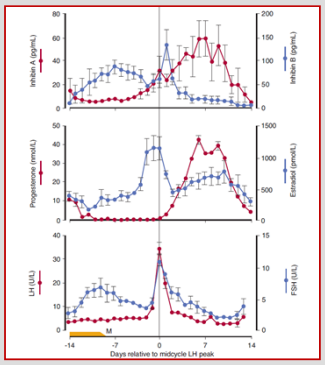


mRNA expression levels of *LHR*, *FSHR*, *CYP19a1* and *AR* in granulosa cells from human immature and mature follicles normalized to the *GAPDH* expression

Follicles: (diameter)	Small antral (≈ 6 mm)	IVM (≈9 mm)	Preovulatory prior to hCG (≈15 mm)	Mature (≈19 mm)	ANOVA
Number	150	14	4	10	*P*-value
LHR (x 1000)	1.3 ± 0.2	1.1 ± 0.6	12.8 ± 3.7	2.8 ±1.1	*P* < 0.00001
FSHR (x 1000)	138 ± 12	29 ± 16	26 ± 12	0.5 ± 0.05	*P* < 0.00001
CYP19a1 (x 1000)	67 ± 13	43 ± 13	1182 ± 164	50 ± 10	*P* < 0.00001
AR (x 1000)	67 ± 8	13 ± 3	12 ± 7	6 ± 0.7	*P* < 0.03

Jeppesen J *et al.*, *JCEM*, 2012;**97**:E1524-31

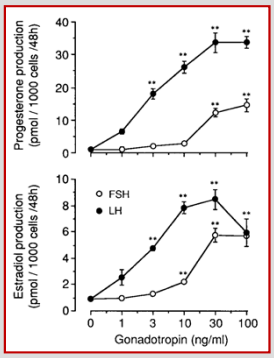
The second half of the follicular phase after selection of the dominant follicle



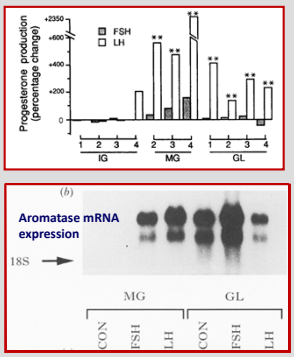
Groome N *et al.*, *JCEM*, 1996;**81**:1401

FSH and LH stimulation of human granulosa cells

From preovulatory follicles prior to the mid-cycle surge

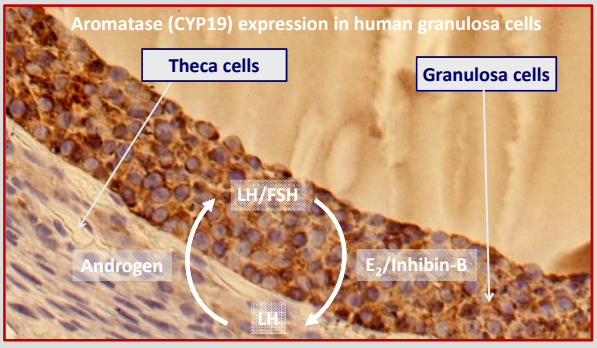


Yong EL, et al. *J. Mol. Endocrinol.* 1994;**12**: 239



Yong EL *et al.*, *JCEM* 1992;**74**:842

LH during the natural menstrual cycle drives estadiol production in the second half of the follicular phase



Acidic FSH isoforms and inhibin-B are important for follicular selection

- Acidic FSH exert a physiological function in promoting androgen production in early/mid follicular phase – via their superior ability to induce inhibin-B production in the granulosa cells, which in synergy with LH stimulate theca cells
- Inhibin-B down-regulate FSH secretion and prepare follicles for the second half of the follicular phase, where LH and FSH in synergy secure oestradiol synthesis

Conclusion

In the first half of the follicular phase acidic FSH isoforms with only a minor contribution from LH

- Stimulate follicular growth
- Stimulate inhibin-B production that in synergy with LH stimulate androgen synthesis in the theca cells
- that up-regulate FSHR expression of the selected follicle, while
- pituitary FSH secretion is down-regulated

In the second half of the follicular phase LH combined with a gradual change to less acidic FSH isoforms

- Stimulates growth of the selected follicle
- Stimulates aromatase expression and oestradiol synthesis
- Stimulates P_4 synthesis and prepare the follicle for ovulation