



# Interrelationships among reproductive hormones and antral follicle count in human menstrual cycles

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## Abstract

It is recognised that ovarian factors, including steroid and protein hormones, are critical in the feedback regulation of pituitary gonadotropins; however, their individual contributions are less defined. The aim of this study was to explore the reciprocal relationships between ovarian and pituitary hormones across the normal ovulatory menstrual cycle as women age. FSH, LH, oestradiol, progesterone, inhibin A, inhibin B and anti-mullerian hormone (AMH) were measured in serum collected every 1–3 days across one interovulatory interval (IOI) from 26 healthy women aged 18–50 years. The antral follicle count (AFC) for follicles 2–5 mm, >6 mm and 2–10 mm were tabulated across the IOI. Independent associations between ovarian hormones/AFC vs pituitary follicle-stimulating hormone (FSH) and luteinising hormone (LH) were investigated using multivariate regression analysis. The data were sub-grouped based on the presence or absence luteal phase-dominant follicles (LPDF). Serum oestradiol and AMH were inversely correlated with FSH in both follicular and luteal phases. Inhibin B correlated inversely with FSH and LH in the late follicular phase and directly in the luteal phase. AFC, inhibin A and progesterone were not key predictors of either FSH or LH. The strong association between AMH and FSH with age implies that AMH, as well as oestradiol and inhibin B are important regulators of FSH. The change in feedback response of inhibin B with both FSH and LH across the cycle suggests two phases of the negative feedback.

## Key Words

- ▶ anti-mullerian hormone
- ▶ inhibin B
- ▶ FSH
- ▶ LH
- ▶ oestradiol

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## Introduction

It is well recognised that ovarian hormones exert a strong inhibitory effect on the pituitary secretion of FSH and LH, as reflected by a 6- to 12-fold increase in the human pituitary secretion of FSH and LH after ovariectomy (1). The key ovarian factors believed responsible are the steroids, oestradiol and progesterone, and the protein

hormones, inhibin A and B. However, their respective roles are not clearly understood.

In a previous study ((2), and (3) designated in this article as the 2009 study), attempts were made to identify the key ovarian hormone factors that are responsible for the feedback regulation of pituitary FSH and LH in



women by exploring their independent associations using a multivariate regression analysis. This study consisted of 76 asymptomatic women aged between 21 and 55 years investigated over one complete menstrual cycle. Serum FSH, LH, oestradiol, progesterone, inhibin A, inhibin B and AMH were measured every 3 days across the cycle. The data were then analysed across the follicular and luteal phases. The results indicated that in the follicular phase, both inhibin B and AMH were inversely associated with FSH and inhibin B with LH. In comparison, in the luteal phase, oestradiol and progesterone were inversely associated with both serum FSH and LH. There appeared to be a 3-day lagged response between inhibin B and FSH. The apparent inverse relationships between ovarian and pituitary hormones were surprising as there is little evidence to support many of these roles, particularly AMH as feedback regulators of FSH and LH.

A number of questions arose from this study:

- (a) Is the close association between AMH and FSH due to the possibility that AMH may directly regulate FSH or act as a proxy for another ovarian factor (perhaps unknown)? Would the association between AMH and FSH weaken if follicle size and number were included as factors in the analysis?
- (b) The AMH ELISA used in the 2009 study had limited sensitivity and was only able to detect serum AMH in 36% of advanced reproductive aged women. Would the availability of more sensitive AMH ELISAs (reported to detect AMH in >95% of samples, (4)) provide a more complete AMH data set?
- (c) To what extent does the presence of hormone-secreting luteal phase-dominant follicles (LPDFs) influence feedback relationships between ovarian and pituitary hormones? It has been previously shown (5, 6) that there are 2–3 follicular waves across each interovulatory interval (IOI). A dominant follicle (i.e.,  $\geq 10$  mm) may develop from any of the waves that emerge during the IOI. In women of reproductive age, only one wave of the IOI has been shown to result in ovulation. In comparison, as women approach menopause, the waves preceding the late follicular phase ovulatory wave of the IOI can result in the development of luteal phase-dominant follicles (LPDFs,  $\geq 10$  mm), which may regress or ovulate. In older women, LPDFs grow larger over a longer period of time, in some cases extending into the subsequent follicular phase.

A recent independent study (7) was undertaken to characterise age-related changes in antral follicle dynamics and hormone production across the IOI. This study consisted of 26 women between the ages of 18 and 50 years. Serum FSH, LH, oestradiol, progesterone, inhibin A, inhibin B, AMH, antral follicle count (AFC) and follicle diameter were determined every 1–3 days across the IOI. Cycles were sub-grouped according to whether they exhibited LPDFs. LPDFs in women of advanced reproductive age were associated with elevated serum oestradiol and inhibin B levels as well as reduced inhibin A and progesterone in the luteal phase (7). These studies have provided to date an increased understanding into age-related associations between follicle diameter, number and hormone production as women age. However, the precise regulatory mechanisms between ovarian vs pituitary hormones as women age remain poorly understood.

The present study provided an opportunity to further explore the relationships between ovarian and pituitary hormones, as a progression from our previous work (2, 3). The inclusion of data to characterise antral follicular dynamics, AFC and AMH (using a more sensitive assay) provided additional insights about the physiological regulation of the human menstrual cycle.

The objectives of the present study were to (a) confirm the previously observed relationships between pituitary and ovarian hormones, (b) clarify the role of additional ovarian factors (follicle diameter and AFC) as possible variables in feedback mechanisms, (c) assess whether a clearer picture of hormone regulation is observed with the introduction of a highly sensitive AMH ELISA and (d) explore whether the presence of a LPDF affects the ovarian–pituitary hormone feedback relationships.

## Methods

The study protocol was approved by the Biomedical Research Ethics Board at the University of Saskatchewan and the Strategic Priorities and Planning Committee of the Saskatoon Health Region. Study procedures were conducted in accordance with the Tri-Council Policy Statement on the Ethical Conduct for Research Involving Humans. Healthy women (aged 18–50,  $n=26$ ) with ovulatory menstrual cycles were recruited from the Saskatoon Health Region, University of Saskatchewan, and the Women's Midlife Health Centre of Saskatchewan. Informed consent was obtained from

all participants before study procedures were initiated. Details of the subjects, study design, hormone measurements, antral follicle dynamics and AFC assessments used in this study have been published previously (7). In brief, healthy women (aged 18–50,  $n=26$ ) with ovulatory menstrual cycles were recruited for this study. Selection criteria and ethics approvals were outlined previously (7). Blood samples were collected every 1–3 days over one IOI. An IOI was defined as the time interval from one ovulation to the subsequent ovulation. Sera were assayed for FSH, LH, oestradiol, progesterone, inhibin A, inhibin B and AMH. AMH was assayed using two different ELISAs: AMH-Gen-II (Beckman Coulter, Chaska, USA) and AMH 24/32 (Anshlabs, Webster, Texas, USA (4)). The AMH 24/32 ELISA is a much more sensitive assay compared to AMH-Gen-II ELISA, enabling the detection of serum AMH in women of advanced reproductive age. The AFC for 3 different diameter categories (i.e., AFC 2–5 mm, 2–10 mm and  $\geq 6$  mm) was quantified using serial transvaginal ultrasonography every 1–3 days across the IOI as previously described (7).

## Analyses

Hormone and follicle data across the IOI were binned into 10 groups. Ovulation 1 (Ov1, days –1, Ov, +1), early luteal phase (ELP, +2, +3, +4), mid-luteal phase (MLP, +5, +6, +7), late luteal phase (LLP, +8, +9, +10) and very late luteal phase (VLLP, +11, +12, +13), menstruation (Men, –1, Men, +1), early follicular phase (EFP, +2, +3, +4), mid follicular phase (MFP, +5, +6, +7), late follicular phase (LFP, +8, +9, +10) and ovulation 2 (OV2, –1, Ov2, +1).

The statistical procedures employed were consistent with those used previously (3) to provide continuity and provide a basis for comparison between studies. The data were analysed initially using multiple regression analysis by assessing the associations between ovarian hormones (FSH and LH) as independent variables and ovarian hormones/AFC as dependent variables. A multivariate regression model with FSH and LH as dependent variables and other ovarian factors as predictors was also developed. Data were evaluated in 3-day windows across the entire follicular phase, in two divisions of the follicular phase termed FP1 (including Men and EFP groups) and FP2 (MLP and LFP groups) and the luteal phase. In some analyses, age was sub-grouped into  $<40$  years and  $\geq 40$  years. The slope coefficients for

the various comparisons were determined following log transformation (referred to as  $\ln\_outcome$ ) of both outcome and predictors. Data transformation permitted a comparison of the magnitude of the slope values between different outcome and predictors. A significant slope value for a given comparison was established when its 95% CIs did not include 0, as previously reported (3). All statistical analyses were conducted using SAS software (v 9.4, 2013; Cary, NC, USA).

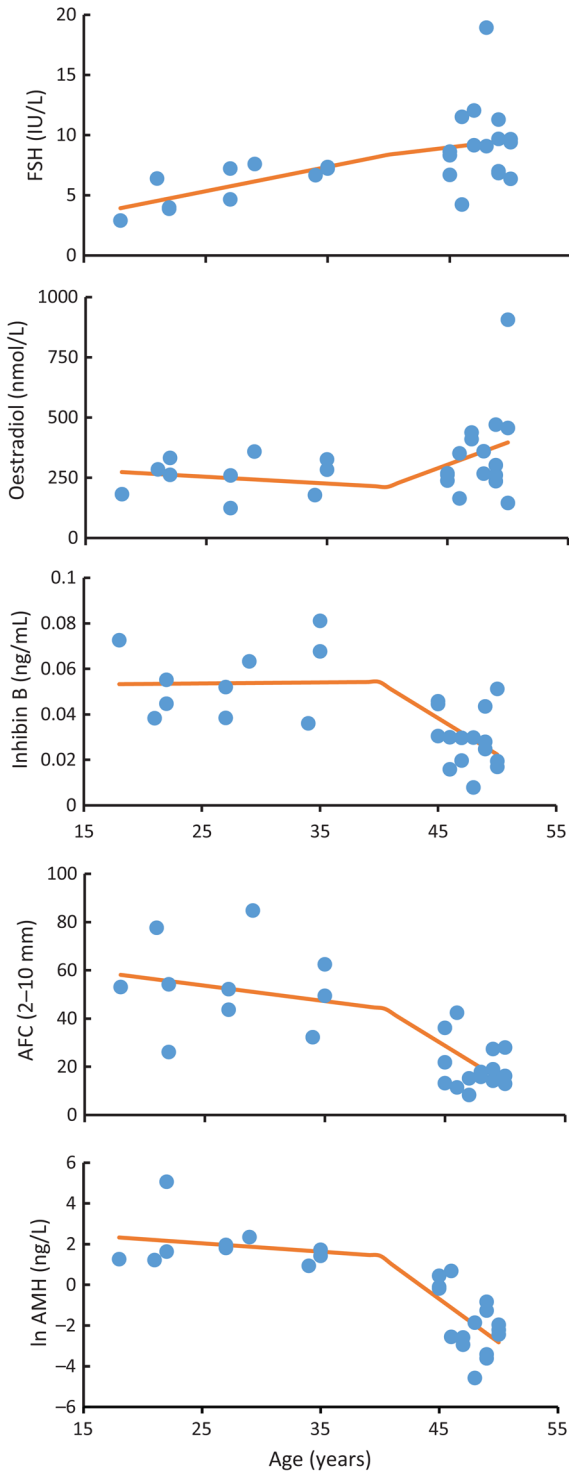
## Results

### Independent relationships between ovarian factors (hormones and antral follicle count) and pituitary factors (FSH and LH)

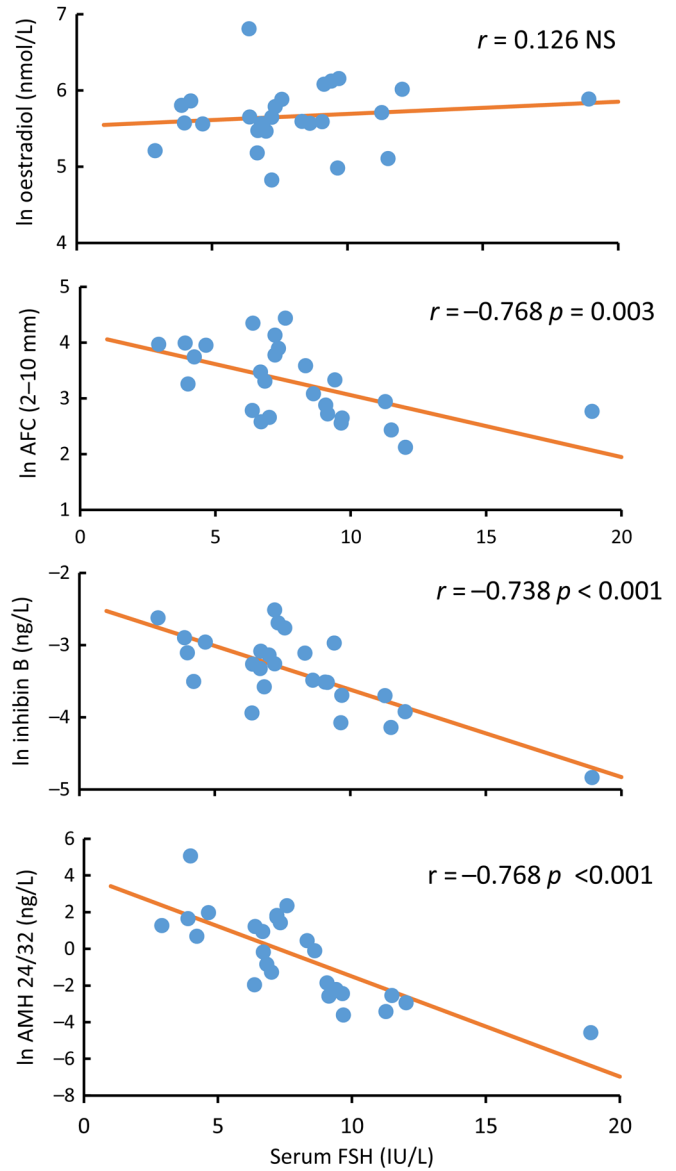
**Associations with age and FSH** Scatter plots between serum FSH, oestradiol, inhibin B, AMH (24/32) and AFC (2–10 mm) vs age are presented in Fig. 1. Using a multivariate model ( $(\ln_{\text{fsh}}, \ln_{\text{lh}}, \ln_{\text{e2}}, \ln_{\text{prog}}, \ln_{\text{inha}}, \ln_{\text{inhb}}, \ln_{\text{amh}} (24-32) \text{ and } \ln_{\text{b2 to 10}}) \sim \text{age}$ ), a direct association was observed between serum FSH and age; inverse associations were noted among inhibin B, AFC (2–10 mm) and AMH (24/32) vs age (Fig. 1). In addition, significant inverse associations were observed among FSH vs inhibin B, AMH (24/32) and AFC (2–10 mm) (Fig. 2). More specifically, as FSH increased with age, inhibin B and AFC (2–10 mm) decreased (Fig. 2). Network plots of these data presenting interactions between the pituitary and ovarian factors in the follicular and luteal phases are presented in Fig. 3.

**Associations across the interovulatory interval** Associations between FSH and LH (as independent variables) vs the ovarian hormones and AFC (as outcome or dependent variables) were assessed across the IOI (Fig. 4) using multi-regression analysis. Significant differences were noted across the cycle with oestradiol inversely proportional to FSH in the luteal phase, inhibin B inversely proportional to FSH and LH in follicular phase and AMH inversely proportional to FSH across the cycle and with LH in the luteal phase.

To assess the associations between ovarian hormones/AFC (as independent variables) vs FSH and LH (as dependent or outcome variables), the data were analysed using multivariate regression methods either across the full follicular phase (Table 1) or within two subdivisions (FP1 and FP2) of the follicular phase (Figs 5 and 6). Significant inverse relationships were observed across the

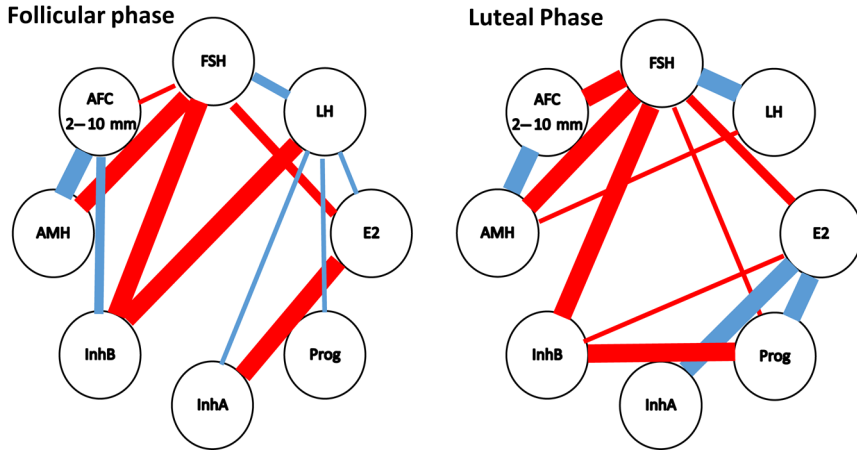


**Figure 1** Representative scatterplots between ovarian and pituitary hormones and age. Serum hormone levels presented were averaged across one interovulatory interval (i.e. between successive ovulation events). The red line on each graph indicates the line of best fit using a piecewise modelling approach. Age is divided into two intervals (before 40 and after 40) with linear regression lines fitted to the data between 0–40 and >40 years.



**Figure 2** Representative scatterplots between ovarian and pituitary hormones. Serum hormone levels were averaged across one interovulatory interval. The red line in each graph is a regression line between X and log Y.

follicular and luteal phases between FSH and oestradiol; however, the significance of this relationship was lost when the follicular phase was sub-grouped into FP1 and FP2 (Figs 5, 6 and Table 1). FSH and LH were inversely related to inhibin B in the late follicular phase but were directly related in the luteal phase. FSH and AMH (24/32) showed an inverse response in both phases of the cycle. LH and AMH were inversely associated in the luteal phase. The patterns between gonadotropins and ovarian hormones were similar when AFC (2–5 mm and >6 mm) was included in the multivariate regression model



**Figure 3** Network plots showing significant associations between serum hormones and antral follicle count (AFC 2–10 mm) in the follicular and luteal phases of the menstrual cycle. Positive and negative correlations are coloured blue and red, respectively. The strength of the correlation between groups is presented as follows; the thick line  $P < 0.001$ , medium thick line  $P < 0.01$ , least thick line  $P < 0.05$ .

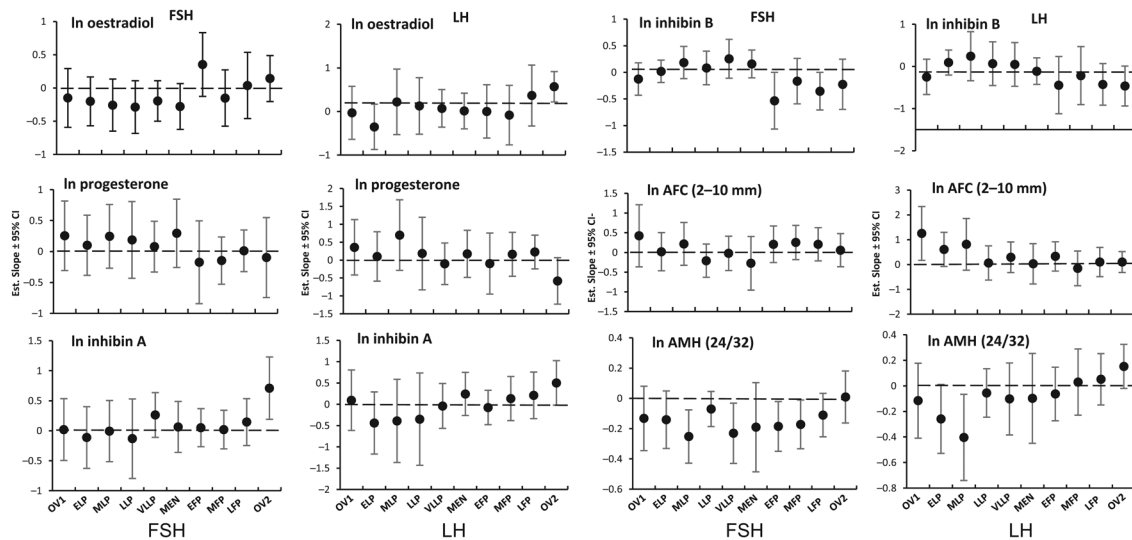
(data not shown). A summary of ovarian and pituitary interactions are presented in Table 2.

**Associations in women with vs without LPDFs** The relationships between ovarian factors and FSH/LH in cycles with and without a LPDF were also assessed (Figs 5 and 6). Generally speaking, similar patterns of association were observed between FSH and the various ovarian factors in the presence or absence of a LPDF across the cycle. In contrast, differences in association between LH and ovarian factors were detected in the presence of a LPDF. Follicular phase LH was negatively associated

with inhibin B in women with LPDFs but not in women without LPDFs. In addition, follicular phase LH correlated positively with AMH and negatively with AFC 2–10 mm in women without LPDFs. The presence of a LPDF did not appear to alter the association between LH and ovarian factors in the luteal phase.

The relationships between FSH/LH and the ovarian hormones, oestradiol, progesterone, inhibin A, inhibin B and AMH-Gen-II were compared in both the 2009 (2, 3) and present studies. In both analyses, the serum levels of all hormones were compared using similar cycle data binning and multivariate regression statistical

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**Figure 4** The independent relationships of ovarian hormones and AFC (2–5 mm and >6 mm) with FSH and LH across the menstrual cycle. The serum hormone and AFC values were binned in 3-day groups across the luteal phase of one cycle beginning at the first ovulation (OV1) and progressing into the follicular phase of the next cycle ending with ovulation (OV2). Slopes and 95% CL are presented. Ovulation 1 (Ov1, days –1, Ov, +1), early luteal phase (ELP, +2, +3, +4), mid-luteal phase (MLP, +5, +6, +7), late luteal phase (LLP, +8, +9, +10) and very late luteal phase (VLLP, +11, +12, +13), menstruation (Men, –1, Men, +1), early follicular phase (EFP, +2, +3, +4), mid follicular phase, (MFP, +5, +6, +7), late follicular phase, (LFP, +8, +9, +10) and ovulation 2 (OV2, –1, Ov2, +1).

**Table 1** Slope values of regression lines between ovarian and pituitary hormones across the follicular and luteal phases of women with normal menstrual cycles for two independent studies; 2009 study (3) and the present analysis.

Study	FSH follicular phase		FSH luteal phase		LH follicular phase		LH luteal phase	
	2009	2015	2009	2015	2009	2015	2009	2015
Oestradiol	-0.35***	-0.22**	0.25***	-0.27**	-0.1	0.1	-0.3	0.07
Progesterone	0.9*	0.056	-0.2***	-0.013	0.2**	0.21	-1.0***	0.018
Inhibin A	-0.05	-0.057	0.1	0.08	0.1*	0.10	0.2	-0.06
Inhibin B	-0.1*	-0.3**	-0.05	0.13**	-0.2**	-0.43***	0.01	0.18*
AMH Gen-II	-0.1***	-0.13***	-0.1***	-0.21***	-0.05	0.055	0.01	-0.1

Significance of regression, \* $P < 0.05$ , \*\* $P < 0.01$  and \*\*\* $P < 0.005$ .

analyses. The slope coefficients and their significances are presented in Table 1. Key observations seen in both studies included: (a) significant inverse associations between FSH vs oestradiol and AMH (Gen-II) in the follicular and luteal phases and (b) an inverse association between FSH and inhibin B in the follicular phase. In contrast, the direct relationship between progesterone and FSH/LH in the follicular phase and inverse relationship in the luteal phase observed in the 2009 study were not noted in the present study.

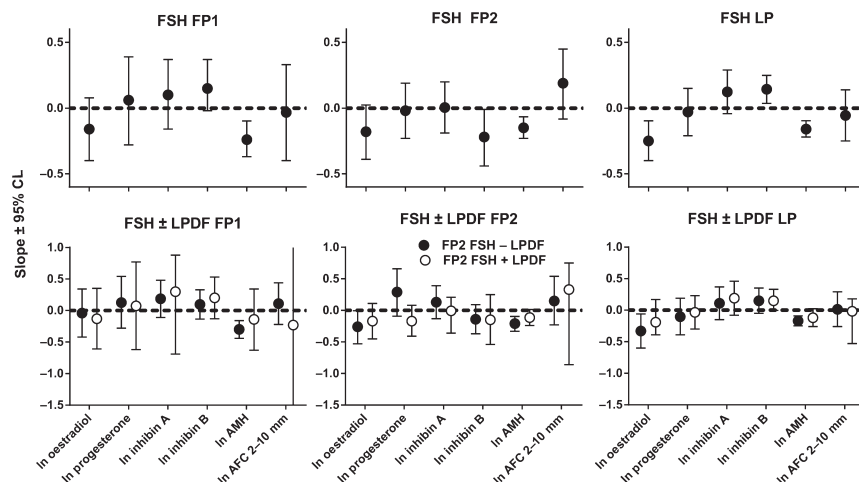
### Discussion

In this study, the relationships between ovarian factors and pituitary hormones were examined in two ways; first, with FSH and LH as independent variables and each ovarian hormone and AFC as outcome or dependent variable; and second, between each ovarian factor (as independent variable) and FSH and LH (as outcome variables). The first approach provides a means to assess the independent relationships between FSH and LH and each ovarian factor; these relationships were assessed

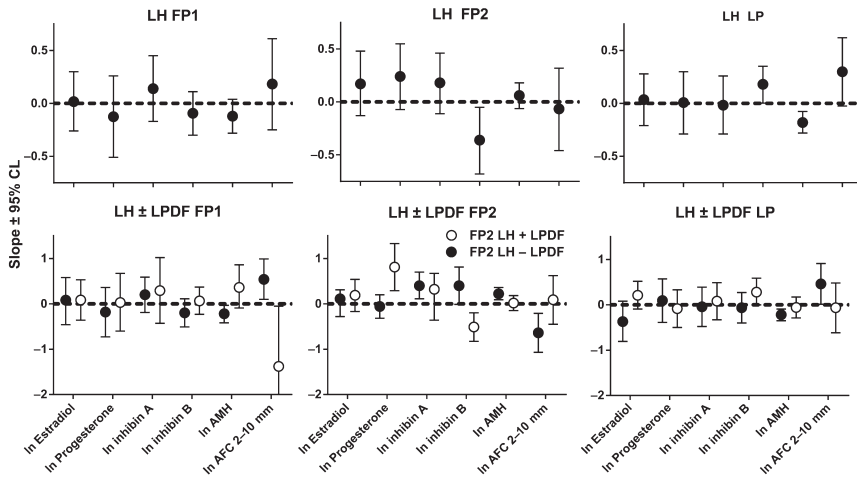
across the menstrual cycle (Fig. 4). The second approach provides a means to identify which of the ovarian factors predict FSH and LH (Figs 5 and 6). Thus, these approaches attempt to differentiate between the effects of individual pituitary hormones on ovarian factors in contrast to the effects of individual ovarian factors on pituitary function.

### Comparison between the 2009 study and the present study

The associations observed between serum ovarian hormones vs FSH and LH showed close agreement between the 2009 study (3) and the present study (Table 2). Inverse relationships were confirmed among oestradiol, AMH and FSH in both follicular and luteal phases of the cycle and between inhibin B and FSH/LH in the follicular phase. However, the inverse relationship observed between serum progesterone and FSH in both phases of the cycle in the 2009 study was not confirmed in the present study. No ready explanation can be provided. In contrast, direct relationships between inhibin B vs FSH and LH in the luteal phase in the present study were novel findings.



**Figure 5** Relationships among ovarian hormones, antral follicle count and FSH. The independent relationships between ovarian hormones and antral follicle count (AFC, 2–10 mm) as outcome variables vs FSH as independent variable in the follicular and luteal phases of the menstrual cycle and those cycles divided into those with and without luteal phase-dominant follicles (LPDFs). Slopes and 95% CL are presented.



**Figure 6** Relationships among ovarian hormones, antral follicle count and LH. The independent relationships between ovarian hormones and antral follicle count (AFC, 2–10 mm) as outcome variables vs LH as independent variable in the follicular and luteal phases of the menstrual cycle and those cycles divided into those with and without luteal phase-dominant follicles (LPDFs). Slopes and 95% CL are presented.

The inclusion of AFC and the more sensitive AMH ELISA has not changed our previous observations that FSH is inversely correlated with oestradiol and AMH across the cycle or that FSH and LH are inversely correlated with inhibin B in the follicular phase.

### Inhibin B and FSH/LH

The inverse relationship between inhibin B and FSH in the follicular phase previously reported in the 2009 study was confirmed in this study; however, the more recent investigations demonstrated that the relationship is confined to the late follicular phase. A similar inverse relationship with LH was less well established but was noted in the 2009 study. It is surprising to note that serum inhibin B but not inhibin A showed a reciprocal

relationship with FSH. This may be explained in part by the temporal changes in inhibin B followed by inhibin A across the follicular phase in relation to FSH. It also should be noted that human inhibin B has higher *in vitro* and *in vivo* bioactivities than human inhibin A, at least in rats (8), which may be reflected in its apparent potent *in vivo* activity in humans. In addition, it is unknown to what extent inhibin B in contrast to inhibin A regulates pituitary LH *in vivo*.

It was also noted that inhibin B was directly correlated with FSH and LH in the luteal phase in contrast to the inverse relationship observed in the follicular phase. One explanation is that as inhibin B levels are low in the luteal phase, it can no longer exert a negative feedback on FSH and LH. One may presume that inhibin B is stimulated by FSH and LH

**Table 2** Summary of relationships between ovarian factors and pituitary hormones in the follicular and luteal phases of the menstrual cycle.

		FSH			LH		
		FP1	FP2	LP	FP1	FP2	LP
All cycles	Estradiol	–	–	Inverse**	–	–	–
	Progesterone	–	–	–	–	–	–
	Inhibin A	–	–	–	–	–	–
	Inhibin B	–	Inverse*	Direct**	–	Inverse*	Direct*
	AMH (24/32)	Inverse***	Inverse***	Inverse***	–	–	Inverse***
	AFC 2–10 mm	–	–	–	–	–	–
Cycles without LPDF	Inhibin B	–	–	–	–	Direct*	–
	AMH (24/32)	Inverse***	Inverse***	Inverse***	Inverse*	Direct**	Inverse***
	AFC 2–10 mm	–	–	–	Direct*	Inverse**	Direct*
Cycles with LPDF	Inhibin B	–	–	–	–	Inverse**	Direct*
	AMH (24/32)	–	–	–	–	–	–
	AFC 2–10 mm	–	–	–	Direct*	–	–

Significance of regression \* $P \leq 0.05$ , \*\* $P < 0.01$  and \*\*\* $P \leq 0.001$ .

AFC, antral follicle count; FP1, follicular phase (menstruation + early FP); FP2, follicular phase (mid + late FP); LP, luteal phase; LPDF, luteal phase dominant follicles.

in the luteal phase, whereas in the follicular phase, the capacity of the growing follicles to produce inhibin B is increased leading to an increased inhibitory feedback action on FSH and LH. This suggests that the same control mechanisms operate in both phases of the cycle. We thus postulate that inhibin B rather than inhibin A plays a role in regulating both FSH and LH, and its reciprocal action is evident in both the follicular and luteal phases. However, it is unclear how inhibin B in contrast to inhibin A exerts its action.

### AMH and FSH

As seen in the 2009 study, serum FSH (as an outcome variable) showed a strong inverse relationship with AMH across the menstrual cycle and with age, suggesting serum AMH was predictive of FSH. This observation was surprising and provided the impetus for the present analysis. The inclusion of a more-sensitive AMH ELISA was intended to establish a more reliable set of AMH values, particularly in the lower ranges. The inclusion of AFC for follicles of various diameters was to establish if AMH was a proxy or substitute for another AFC factor based on the very high correlation between serum AMH and AFC (4). The inclusion of AFC did not change the independent inverse association of AMH and FSH. There is limited evidence available to substantiate any postulated age-related feedback role of AMH on FSH. AMH has been shown to inhibit FSH $\beta$  subunit synthesis in a mouse anterior pituitary (L $\beta$ T2) cell line and rat pituitary cells *in vitro* (9); presumably, this is likely although not yet shown in the human pituitary. By comparison, there is no evidence that FSH stimulates AMH synthesis by the ovary (10), a key component in any ovarian:pituitary negative feedback mechanism.

AMH is produced by granulosa cells from gonadotropin-unresponsive preantral follicles under stimulation by oocyte-specific factors (e.g. BMP2, 6, 7, 15, GDF9) from the oocyte (11, 12). AMH has been shown to inhibit the progression of primordial to preantral follicles (13, 14, 15) in mice but stimulates progression in primate monkeys (16). AMH has been shown to inhibit FSH-induced oestradiol production by human and primate granulosa cells (16, 17, 18, 19, 20). Moreover, oestradiol has been shown to inhibit AMH synthesis *in vitro* suggesting an indirect action of FSH on AMH synthesis (20).

It is hypothesised that as AFC and serum AMH decrease with age, FSH and oestradiol production by

FSH-sensitive follicles increase (3). Oestradiol is thought to exert an inhibitory role on FSH, whereas the reduced AMH levels may lead to elevated FSH levels. It would appear that if oestradiol and AMH are two of the major factors in regulating FSH, the effects of AMH are more pronounced with age. It is recognised that as oestradiol exerts its inhibitory role at the hypothalamus, whereas AMH is probably at the pituitary, different biological actions are likely involved. AMH and oestradiol have been shown to be reciprocally related in follicular fluid (15, 21, 22).

A number of clinical studies have observed an inverse relationship between serum FSH and AMH (23, 24, 25, 26, 27). However, with the exception of Pigny (26), these analyses did not differentiate AMH's role from the effects of other ovarian feedback factors. The studies of Pigny (26) investigated the feedback relationship between AMH and FSH in women with polycystic ovarian syndrome (PCOS) where serum AMH levels were elevated. A significant inverse relationship has been observed between serum AMH and FSH in both PCOS and control women; however, this association between AMH and other ovarian hormones vs FSH was lost following multivariate regression analysis. We hypothesise that these studies do not exclude an inverse relationship between AMH and FSH but that owing to the multiple interactions between ovarian and pituitary hormones, an in-depth analysis of ovarian and pituitary hormones in PCOS women similar to this study is required to clarify this relationship.

In summary, we hypothesise that from early stages in reproductive life, AMH, produced by the preantral/antral follicles, suppresses FSH secretion using similar intracellular TGF $\beta$  inhibitory pathways to that observed with inhibin B and that this inhibition decreases in parallel with the decrease in folliculogenesis with age. The role of AMH within the ovary is important in regulating the release of follicles from the preantral pool.

It is interesting that including AFC as a variable had minimal effect on the multivariate regression analysis. Its inclusion was introduced to identify if AFC (as a surrogate or proxy for other ovarian factors) would also show a reciprocal relationship with FSH. We conclude that probably all the ovarian factors involved in these interactions with FSH have been identified. The inverse relationship observed between AFC (2–10mm) and FSH is attributed to the close relationship between AFC (2–10mm) and AMH, with AMH as the key factor.



## Luteal phase-dominant follicles

The presence of dominant follicles in the luteal phase were associated with elevated oestradiol, inhibin B and AFC (>6mm) (6, 7), which may have resulted in changes to their feedback actions on FSH and LH. In this study, we showed that the inverse associations between FSH vs oestradiol and AMH were similar in women with and without a LPDF. The presence of a LPDF resulted in altered relationships between LH and the other ovarian hormones and AFC, and further studies with a larger sample size are required to confirm these findings. The impact of the presence of LPDF on ovarian:pituitary feedback mechanisms is unclear. However, it has been shown that LPDF cycles are associated with elevated and prolonged oestradiol patterns (7), particularly with women of advanced reproductive age, which may be a consequence of this reduction in ovarian control of pituitary FSH and LH secretion.

## Summary

We now postulate the following:

- AMH is independently and inversely correlated with FSH but not LH, and probably plays a role in conjunction with oestradiol (and to a less extent, inhibin B) in regulating FSH across the menstrual cycle throughout adult reproductive life.
- Inhibin B plays an important role in both the follicular and luteal phases in regulating FSH and LH.
- Oestradiol specifically regulates FSH throughout the cycle.
- The inclusion of AFC in the analysis provided little additional information, suggesting that the key ovarian factors have been identified.
- The impact of the presence of a LPDF on ovarian:pituitary feedback mechanisms is unclear. Further studies are required to clarify their involvement.

### Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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## References

- Yen SSC & Tsai CC. The effect of ovariectomy on gonadotropin release. *Journal of Clinical Investigation* 1971 **50** 1149–1153. (doi:10.1172/JCI106587)
- Hale GE, Hughes CL, Burger HG, Robertson DM & Fraser IS. Atypical estradiol secretion and ovulation patterns caused by luteal out-of-phase (LOOP) events underlying irregular ovulatory menstrual cycles in the menopausal transition. *Menopause* 2009 **16** 50–59. (doi:10.1097/gme.0b013e31817ee0c2)
- Robertson DM, Hale GE, Jolley D, Fraser IS, Hughes CL & Burger HG. Interrelationships between ovarian and pituitary hormones in ovulatory menstrual cycles across reproductive age. *Journal of Clinical Endocrinology and Metabolism* 2009 **94** 138–144. (doi:10.1210/jc.2008-1684)
- Robertson DM, Kumar A, Kalra B, Shah S, Pruyssers E, Vanden Brink H, Chizen D, Visser JA, Themmen AP & Baerwald A. Detection of serum antimüllerian hormone in women approaching menopause using sensitive anti-müllerian hormone enzyme-linked immunosorbent assays. *Menopause* 2014 **21** 1277–1286. (doi:10.1097/GME.0000000000000244)
- Baerwald A, Adams G & Pierson R. A new model for ovarian follicular development during the human menstrual cycle. *Fertility and Sterility* 2003 **80** 116–122. (doi:10.1016/S0015-0282(03)00544-2)
- Vanden Brink H, Chizen D, Hale G & Baerwald A. Age-related changes in major ovarian follicular wave dynamics during the human menstrual cycle. *Menopause* 2013 **20** 1243–1254. (doi:10.1097/GME.0b013e31828cfb62)
- Vanden Brink H, Robertson DM, Lim H, Lee C, Chizen D, Harris G, Hale G, Burger H & Baerwald A. Associations between antral ovarian follicle dynamics and hormone production throughout the menstrual cycle as women age. *Journal of Clinical Endocrinology and Metabolism* 2015 **100** 4553–4562. (doi:10.1210/jc.2015-2643)
- Makanji Y, Temple-Smith PD, Walton KL, Harrison CA & Robertson DM. Inhibin B is a more potent suppressor of rat follicle-stimulating hormone release than inhibin a in vitro and in vivo. *Endocrinology* 2009 **150** 4784–4793. (doi:10.1210/en.2008-1783)
- Bédécarrats GY, O'Neill FH, Norwitz ER, Kaiser UB & Teixeira J. Regulation of gonadotropin gene expression by Müllerian inhibiting substance. *PNAS* 2003 **100** 9348–9353.
- Wachs DS, Coffler MS, Malcom PJ & Chang RJ. Serum anti-müllerian hormone concentrations are not altered by acute administration of follicle stimulating hormone in polycystic ovary syndrome and normal women. *Journal of Clinical Endocrinology and Metabolism* 2007 **92** 1871–1874.
- Ogura-Nose S, Yoshino O, Osuga Y, Shi J, Hiroi H, Yano T & Taketani Y. Anti-Müllerian hormone (AMH) is induced by bone morphogenetic protein (BMP) cytokines in human granulosa cells. *European Journal of Obstetrics, Gynecology and Reproductive Biology* 2012 **164** 44–47. (doi:10.1016/j.ejogrb.2012.05.017)
- Gilchrist RB, Lane M & Thompson JG. Oocyte-secreted factors: regulators of cumulus cell function and oocyte quality. *Human Reproduction Update* 2008 **14** 159–177. (doi:10.1093/humupd/dmm040)
- Durlinger AL, Visser JA & Themmen AP. Regulation of ovarian function: the role of anti-Müllerian hormone. *Reproduction* 2002 **124** 601–609. (doi:10.1530/rep.0.1240601)
- Durlinger AL, Grijters MJ, Kramer P, Karels B, Ingraham HA, Nachtigal MW, Uilenbroek JT, Grootegoed JA & Themmen AP. Anti-Müllerian hormone inhibits initiation of primordial follicle growth in the mouse ovary. *Endocrinology* 2002 **143** 1076–1084.

- 15 Nilsson E, Rogers N & Skinner MK. Actions of anti-Müllerian hormone on the ovarian transcriptome to inhibit primordial to primary follicle transition. *Reproduction* 2007 **134** 209–221. (doi:10.1530/REP-07-0119)
- 16 Xu J, Bishop CV, Lawson MS, Park BS & Xu F. Anti-Müllerian hormone promotes pre-antral follicle growth, but inhibits antral follicle maturation and dominant follicle selection in primates. *Human Reproduction* 2016 **31** 1522–1530. (doi:10.1093/humrep/dew100)
- 17 Grossman MP, Nakajima ST, Fallat ME & Siow Y. Müllerian-inhibiting substance inhibits cytochrome P450 aromatase activity in human granulosa lutein cell culture. *Fertility and Sterility* 2008 **89** 1364–1370. (doi:10.1016/j.fertnstert.2007.03.066)
- 18 Pellatt L, Rice S, Dilaver N, Heshri A, Galea R, Brincat M, Brown K, Simpson ER & Mason HD. Anti-Müllerian hormone reduces follicle sensitivity to follicle-stimulating hormone in human granulosa cells. *Fertility and Sterility* 2011 **96** 1246–1251. (doi:10.1016/j.fertnstert.2011.08.015)
- 19 Visser JA & Themmen AP. Role of anti-Müllerian hormone and bone morphogenetic proteins in the regulation of FSH sensitivity. *Molecular and Cellular Endocrinology* 2014 **382** 460–465. (doi:10.1016/j.mce.2013.08.012)
- 20 Grynberg M, Pierre A, Rey R, Leclerc A, Arouche N, Hesters L, Catteau-Jonard S, Frydman R, Picard JY, Fanchin R, *et al.* Differential regulation of ovarian anti-müllerian hormone (AMH) by estradiol through  $\alpha$ - and  $\beta$ -estrogen receptors. *Journal of Clinical Endocrinology and Metabolism* 2012 **97** E1649–E1657. (doi:10.1210/jc.2011-3133)
- 21 Andersen CY & Byskov AG. Estradiol and regulation of anti-Müllerian hormone, inhibin-A, and inhibin-B secretion: analysis of small antral and preovulatory human follicles' fluid. *Journal of Clinical Endocrinology and Metabolism* 2006 **91** 4064–4069. (doi:10.1210/jc.2006-1066)
- 22 Andersen CY, Schmidt KT, Kristensen SG, Rosendahl M, Byskov AG & Ernst E. Concentrations of AMH and inhibin-B in relation to follicular diameter in normal human small antral follicles. *Human Reproduction* 2010 **25** 1282–1287. (doi:10.1093/humrep/deq019)
- 23 van Rooij IA, Broekmans FJ, te Velde ER, Fauser BC, Bancsi LF, de Jong FH & Themmen AP. Serum anti-Müllerian hormone levels: a novel measure of ovarian reserve. *Human Reproduction* 2002 **17** 3065–3071. (doi:10.1093/humrep/17.12.3065)
- 24 Seifer DB, MacLaughlin DT, Christian BP, Feng B & Shelden RM. Early follicular serum müllerian-inhibiting substance levels are associated with ovarian response during assisted reproductive technology cycles. *Fertility and Sterility* 2002 **77** 468–471. (doi:10.1016/S0015-0282(01)03201-0)
- 25 de Vet A, Laven JS, de Jong FH, Themmen AP & Fauser BC. Antimüllerian hormone serum levels: a putative marker for ovarian aging. *Fertility and Sterility* 2002 **77** 357–362. (doi:10.1016/S0015-0282(01)02993-4)
- 26 Pigny P, Merlen E, Robert Y, Cortet-Rudelli C, Decanter C, Jonard S & Dewailly D. Elevated serum level of anti-müllerian hormone in patients with polycystic ovary syndrome: relationship to the ovarian follicle excess and to the follicular arrest. *Journal of Clinical Endocrinology and Metabolism* 2003 **88** 5957–5962. (doi:10.1210/jc.2003-030727)
- 27 Lie Fong S, Visser JA, Welt CK, de Rijke YB, Eijkemans MJ, Broekmans FJ, Roes EM, Peters WH, Hokken-Koelega AC, Fauser BC, *et al.* Serum anti-müllerian hormone levels in healthy females: a nomogram ranging from infancy to adulthood. *Journal of Clinical Endocrinology and Metabolism* 2012 **97** 4650–4655. (doi:10.1210/jc.2012-1440)

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