

Dynamics of FSH-induced follicular growth in subfertile women: relationship with age, insulin resistance, oocyte yield and anti-Mullerian hormone

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BACKGROUND: During excess FSH treatment, different categories of follicles can be discerned: those responding and appearing to grow immediately (FolsS8) and those appearing subsequently during the follicular phase (Fols/d). These follicular categories were explored in cycles of assisted reproduction in the context of follicular biology, including primordial follicle pool (PFP) depletion, age, insulin resistance and potential markers. **METHODS:** Follicular cohorts were examined in 365 conventional ART cycles and related to patient insulin sensitivity, plasma FSH and anti-Mullerian hormone (AMH). **RESULTS:** Age had no influence upon the FolsS8 category but was associated with a significant ($P < 0.005$) decline in the Fols/d. In contrast, insulin-resistant polycystic ovary syndrome (IR-PCOS) showed a significant ($P = 0.005$) increase in FolsS8. Circulating AMH correlated strongly with oocyte yield and Fols/d. **CONCLUSION:** Age showed little impact on the initial follicular cohort, but a significant impact upon the secondary cohort, while insulin resistance appeared to promote the former category alone. The disturbance to follicular dynamics and AMH in IR-PCOS reflected a larger stockpile of FSH-sensitive follicles. Circulating AMH appears to represent all categories of antral follicles observed.

Key words: AMH/follicle cohort/FSH/oocyte yield/PCOS

Introduction

It is conventional practice to stimulate multiple follicular growth to obtain multiple oocytes in cycles of assisted reproduction (ART, IVF and ICSI). This is effected using exogenous FSH in daily doses, leading to supranormal circulating concentrations and recruitment of all follicles whose FSH sensitivity threshold is exceeded (Baird, 1983). The dynamics of follicular growth and the influence of associated factors can be explored using observations made in these cycles.

Despite the presence of FSH receptors from the earliest stages of development, follicles only develop sensitivity to FSH as one of the last developmental stages after many weeks of growth from the primordial stage (Gougeon, 1998). The small number doing so (at the 2–5 mm antral stage) in any 1 day depends upon a number of factors. Normally, these follicles are either recruited by the perimenstrual rise in FSH, or they undergo atresia due to FSH deprivation (Gougeon, 1996; Hussein, 2005). The factors potentially influencing the number of viable antral follicles attaining FSH sensitivity include the size of the primordial follicle pool (PFP) and also factors promoting or controlling follicular growth and survival such as members of the TGF- β family (Hussein, 2005). The model of follicular life described by Faddy

(2000) shows that in young females, the main point of follicular attrition is at the very earliest stage of development, the primordial-to-primary transition, whereas at older ages a higher proportion of follicles leaving the PFP achieve the antral stage development. The growth, survival and development of these follicles may be influenced by insulin-related growth factors, as patients with polycystic ovary syndrome (PCOS), with their intrinsic insulin hypersecretion, show excessive number of follicles growing through to antral stages. This ‘stockpiling’ process leads to the diagnostic anovulation (Jonard and Dewailly, 2004). Maciel *et al.* (2004) recently described the stockpiling process of follicles at both immature and advanced stages of development in women with PCOS, again suggesting that insulin-related factors influence the categorization of follicular development, which may be effected by attenuating the normal rate of development.

Anti-Mullerian hormone (AMH, Mullerian-inhibiting substance) is expressed and secreted by granulosa cells in most follicular stages, including maximal expression in small antral follicles (Laven *et al.*, 2004). In theory, it may be used as a marker of the total developing follicular cohort before FSH-induced growth, and it has been explored in the IVF setting and in polycystic ovary syndrome (PCOS), where changes in the follicular profile can be observed.

As follicles grow, the number of granulosa cells increases, and markers of granulosa cell activity can be used to indicate follicular development. However, factors such as estradiol (E_2) and inhibin-B are subject to numerous control mechanisms which limit their usefulness in clinical terms, either to detect stages of follicular development or to predict responses to excessive FSH, as in ART cycles. In this latter case, alternative methods are commonly employed, including perimenstrual FSH values, ovarian volume and antral follicle count (Seifer *et al.*, 2002; Muttukrishna *et al.*, 2005).

When exogenous FSH is administered, the number of follicles induced to grow depends exclusively upon the number of follicles arriving at the end of their protracted development phase and the number of follicles attaining FSH sensitivity. The size of this viable antral follicle pool will depend on the number attaining FSH sensitivity each day and the number losing the ability to respond to FSH (undergoing atresia). The dynamics of these follicle cohorts can be estimated by observations made during a cycle stimulated by excessive FSH doses, as in conventional ART cycles, by counting: (1) the number of follicles appearing after a given period of stimulation, and/or, (2) the increase in the number of follicles observed between two time points after a period of FSH stimulation when the steady-state concentrations have been established. Estimate (1) should reflect the size of the viable, FSH-sensitive antral follicle pool at the start of FSH stimulation. It would be subject to the theoretical variables of degree of suppression of FSH by the GnRH agonist, the variable duration required to attain steady-state concentrations of supranormal FSH in the circulation and the variation in the threshold FSH sensitivity of the follicles. Estimate (2) is calculated only after the establishment of the steady-state FSH concentrations and also after the recruitment of the first stockpile of follicles, and therefore should reflect the number of follicles attaining FSH sensitivity on a daily basis (entering the FSH-sensitive zone) and suffers only from technical errors and variations in FSH sensitivity.

Analyses of the two measurements can be used to explore relationships of follicular categories with potential markers of follicular development such as AMH and also factors influencing these relationships, such as age and insulin sensitivity.

The aim of this study was to record the appearance of growing antral follicles under FSH drive during ART cycles using the two measures described and relate these figures to the estimates of the number of follicles leaving the PFP, the perimenstrual FSH and AMH concentrations and the yield of oocytes at oocyte retrieval. The variables in the follicle cohorts were explored by assessing the number of medium-sized follicles appearing in the ovaries by stimulation day 8 (S8) and also the number of follicles appearing per day after the steady-state FSH concentration is exceeded. The responses of insulin-resistant women with PCOS were also explored within this paradigm.

Methods

Patients and treatment

Successive patients undergoing their first ART treatment cycle ($n = 365$) were down-regulated with the depo GnRH agonist (Prostap, Wyeth, Maidenhead, UK) initiated on cycle day 21. Stimulation commenced

2 weeks later, when the circulating E_2 was <100 pg/ml, combined with a thin endometrium, and no ovarian cysts on transvaginal ultrasound scan. Ovarian stimulation was effected with exogenous gonadotrophins in the form of either Menogon (Ferring Pharmaceuticals, Langley, UK) or Gonal-F (Serono, Feltham, UK). The starting daily dose of FSH was determined by age, whereby women of <37 years received 225 IU and those >36 years received 300 IU each day. Ovarian follicular responses were monitored with serum E_2 concentrations and transvaginal ultrasonic assessment of follicular growth. The first response scan was performed on S8, providing the FolsS8 value, and subsequent scans were performed according to the S8 response. The number of oocytes following oocyte retrieval was also recorded.

Assays

One month before treatment, a perimenstrual blood sample (cycle day 2–5) was taken for assay of FSH and sex hormone-binding globulin (SHBG), as an indicator of insulin resistance and also AMH. The FSH and SHBG concentrations in peripheral plasma were estimated routinely using the Immulite semi-automated assay system (DPC, Los Angeles, CA, USA).

The AMH assay was performed in batches using the ELISA assay provided by DSL (Webster, TX, USA), with values presented in concentrations of pmol/l (conversion factor to pmol/l = ng/ml \times 7.143).

Ultrasonography

Estimates of the viable antral follicle category size (number of follicles attaining FSH sensitivity), within a given time frame, were obtained from ovarian ultrasound identification of follicles whose diameter was ≥ 12 mm at two specific time points. The critical diameter (12 mm) was determined before the study on the basis of previous observations that the minimum size of lead follicle at S8 was 12 mm in $>85\%$ of cycles. Correspondingly, the database was set up to record the number of such follicles present, representing the pool of FSH sensitive follicles present at the start of stimulation. The two values determined and explored were (1) the number of such follicles appearing at S8 (FolsS8) and (2) the number of such follicles appearing between S8 and the subsequent scan (S10 to S12) divided by the number of days. This latter value was deemed to represent an estimate of the number of follicles attaining FSH sensitivity per day (Fols/d).

Definitions

FolsS8. The stockpile of follicles responding to exogenous FSH was estimated by counting the number of follicles ≥ 12 mm determined on S8.

Fols/d. The number of follicles attaining FSH sensitivity each day was defined as the increase in the number of follicles with diameter ≥ 12 mm appearing between S8 and the following scan (S10 to S12) divided by the number of days between the scans.

Excess response to FSH in ART cycles was defined as a yield of ≥ 20 oocytes, whereas 'poor responses' were defined by either discontinuation of treatment because of insufficient follicles or an oocyte yield of ≤ 2 .

Insulin-resistant PCOS (IR-PCOS) was diagnosed when a patient showed oligomenorrhoea combined with a circulating SHBG concentration of ≤ 35 nmol/l. The use of SHBG as a surrogate marker for insulin resistance was justified by Nestler, 1993.

The primordial follicle pool. Estimates of the number of follicles leaving the PFP per day for particular age groups were obtained from the model presented by Faddy (2000).

Variables according to age. Values of the variables FolsS8 and Fols/d according to age were determined by calculating the mean of the

variable for all patients within a range set at 2 years either side of the given age point.

Statistics

The distribution of groups of variable data was assessed, and Gaussian or non-Gaussian distributions were treated appropriately. Changes in variables over the age ranges were assessed using analysis of variance (Kruskal–Wallis ANOVA test). Comparisons of insulin-resistant PCOS with normal were effected using the Mann–Whitney *t*-test. Correlation (Spearman *r*-test) and linear correlation assessments were effected to determine the relationships between the parameters. Step-wise correlation evaluations were effected for oocyte yields and AMH. The statistics package used for these analyses was Minitab (version 13, for windows; Minitab®, State College, PA). Significance was determined when $P \leq 0.05$.

Results

Baseline patient information

The baseline information on the patients was probably representative of those attending most ART clinics. Their mean age was 33.6 years (95% confidence limits (CL) = 33.59 and 33.56, SD = 4.5), and their mean BMI was 24.4 (CL = 24.41 and 24.44 kg/m², SD = 3.1). The proportion defined as having insulin-resistant PCOS was not large (28 cases from a total of 362 cases). Concentrations of AMH showed a non-Gaussian distribution with median value of 10.0 pmol/l and a mean of 13.9 pmol/l (SD = 15, CL = 12.5 and 16.0).

The influence of age

Table I summarizes the ranges of values according to age, within groups from 25 to 40 years. They showed the expected significant declines in oocyte yield and AMH. However, the significant decline in the Fols/d value was entirely dependent upon the high numbers in the 25-year group, as in the absence of this group, the remaining data showed no age-related change ($P = 0.60$, Kruskal–Wallis ANOVA). FSH showed modest increases over the same time scale, but the number of follicles observed at S8 (FolsS8) showed no significant change over the age range.

It is noteworthy that in the 40-year-old age group, the estimate of Fols/d (1.9) was close to the PFP daily-depletion rate ($n = 2.7$) estimated from the model described by Faddy (2000). This contrasts with the data from the 25-year-old where the number of follicles leaving the PFP is four-fold higher than the number recruited by FSH (Figure 1).

Figure 1 shows the estimates of the categories of follicles according to age and the circulating AMH concentrations. The PFP-depletion value is an estimate of the number of follicles leaving the PFP on a daily basis (Faddy, 2000), which is a reflection of the basic ovarian reserve. The data show that there are profound changes in the depletion of the PFP over the age range examined, but there is negligible change in the number of follicles attaining FSH sensitivity each day over that same age range. The profile of AMH follows that of the recruitable follicles rather than the number of follicles leaving the PFP, indicating that AMH is reflective of the terminal growing follicle cohort rather than the absolute ovarian reserve.

The influence of BMI

It is clear from Table II that BMI showed only the weakest correlation analyses with any of the parameters. Its (weakly) significant relationship with AMH probably reflects the contribution from patients with PCOS, who are generally overweight. It is notable that BMI had no influence over either initial (FolsS8) or secondary responses to FSH (Fols/d).

Initial responses to FSH represented by FolsS8

The FolsS8 value, representing the size of the follicle cohort with the ability to respond to FSH at the start of stimulation, showed a mean value of 3.4 follicles present, which had generally weak correlations with the other parameters (Table II). It showed no relationship with BMI and a significant but weak correlation with age. Surprisingly, its ability to predict the Fols/d value, although significant, was also weak, while its correlations with oocyte yield, FSH and AMH concentrations were more substantial. There were 52 cases who subsequently received HCG and where no follicles of these dimensions were recorded on S8. These cases showed normal oocyte yields (8.0 ± 6.4), normal AMH ($12.1 + 10$ pmol/l) and had a mean age of 34 years. Correspondingly, this evaluation of ovarian response had a poor predictive power.

Subsequent follicular recruitment rate (Fols/d)

The Fols/d measure, deemed to represent the number of follicles responding to FSH after the initial recruitment phase, showed a mean value of 2.2 follicles per day. The value showed strong relationships with both oocyte yield and circulating AMH concentrations. It showed a stronger goodness of

Table I. Mean values (SE) for ovarian response factors and FSH concentrations in cycles of assisted conception according to age groups

	Age (± 2 years)				P (ANOVA) (Kruskal–Wallis)
	25	30	35	40	
ART cases (<i>n</i>)	26	72	124	56	
FolsS8	4.8 (0.78)	3.4 (0.39)	3.4 (0.29)	2.8 (0.35)	0.161
Fols/d	3.2 (0.33)	2.2 (0.20)	2.1 (0.14)	1.9 (0.18)	0.005
Oocyte yield ^a	14.9 (1.9)	10.9 (0.84)	9.0 (0.55)	8.8 (0.76)	<0.001
FSH (IU/l)	6.4 (0.34)	7.9 (0.45)	8.3 (0.29)	8.5 (0.41)	0.006
AMH (pmol/l)	21.6 (2.7)	14.1 (1.6)	13.7 (1.1)	9.0 (0.76)	<0.001

AMH, anti-Mullerian hormone.

^aOnly those cases receiving HCG.

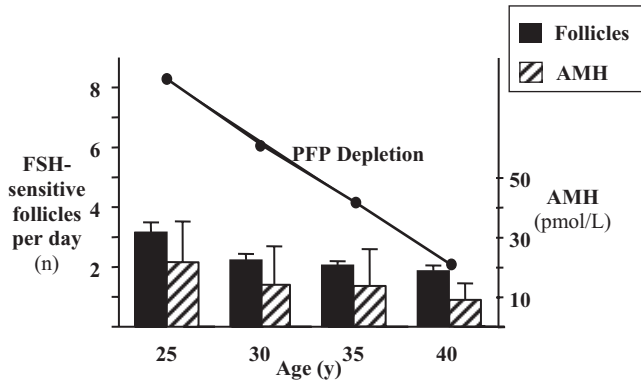


Figure 1. Profiles of the number of follicles leaving the primordial follicle pool (PFP depletion) and those attaining FSH sensitivity on a daily basis (Fols/d) according to age, set against the circulating anti-Mullerian hormone (AMH) concentration.

Table II. Linear regression assessments of goodness of fit values (r^2) and significance values (lower left figure) of the investigated parameters relative to each other

	Age	BMI	FolsS8	Fols/d	Oocytes	AMH	FSH
Age	–	0.007	0.035	0.061	0.078	0.116	0.046
BMI	0.152	–	0.003	0.008	0.008	0.015	0.012
FolsS8	0.001	0.38	–	0.015	0.124	0.109	0.099
Fols/d	<0.001	0.15	0.018	–	0.292	0.289	0.062
Oocytes	<0.001	0.11	<0.001	<0.001	–	0.362	0.117
AMH	<0.001	0.035	<0.001	<0.001	<0.001	–	0.089
FSH	<0.001	0.053	<0.001	<0.001	<0.001	<0.001	–

AMH, anti-Mullerian hormone.

fit value (r^2) for age than did FolsS8. This suggests that age (within the range explored) influences the number of follicles attaining FSH sensitivity per day more directly than it does the size of the stockpile represented by FolsS8.

Oocyte yield

The relationship between the oocyte yield in those cases undergoing oocyte pickup (mean value = 10.0 ± 6.9) and the other parameters was also examined in Table II. There were significant but weak correlations with age and FSH. Both Fols/d and AMH showed strong predictive (r^2) values indicating close relationships and high predictive capacity of these two elements. This observation also demonstrates the importance of the contribution of the second phase of follicles to the complement of oocytes in a standard IVF cycle. The ability of AMH to predict the oocyte yield was effectively three-fold higher than that of FSH.

A stepwise regression analysis of oocyte yield versus AMH, FolsS8, Fols/d and FSH showed that the combined model was predictive to 46% ($r^2 = 46.3$, adjusted = 45.6). However, AMH contributed the overwhelming proportion of this predictive capacity ($r^2 = 33.0$, adjusted = 32.8).

AMH and its predictive value in ART cycles

Table II summarizes that AMH has highly significant relationships with all the parameters examined except BMI, with the

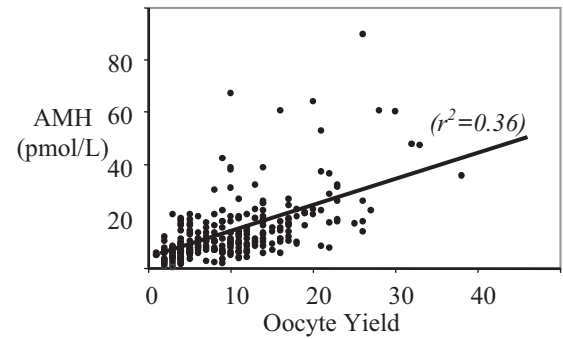


Figure 2. Correlation between circulating anti-Mullerian hormone (AMH) and oocyte yield after standard controlled ovarian stimulation.

two strongest r^2 values applying to Fols/d (29%) and oocyte yield (36%). This suggests that much of the circulating AMH is secreted by viable follicles which are able to respond positively to FSH stimulation.

A stepwise regression analysis of AMH versus oocyte yield, Fols/d, FolsS8, age and FSH revealed that FSH did not qualify for inclusion ($\alpha = 0.15$) and that the combined model had a predictive power of 42% ($r^2 = 42.2$, adjusted = 41.3). Oocyte yield contributed the overwhelming proportion of this predictive capacity ($r^2 = 33.0$, adjusted = 32.8).

In practical terms, the ability of AMH to predict the number of follicles growing in response to FSH and the oocyte yield in ART cycles is clearly strong, as exemplified by the 10-fold difference in values for the excess responses and poor responses defined above. Excess responses ($n = 32$) showed a median value of AMH of 28.3 pmol/l (confidence limits of 26.6 and 46.5), while poor responses ($n = 51$) showed a median value of AMH of 2.9 pmol/l (confidence limits of 2.4 and 4.3).

There were 23 cases where the circulating AMH concentration before treatment was ≤ 1.0 pmol/l of which 19 (83%) were discontinued before oocyte retrieval for lack of response.

The scatter plot relating circulating AMH to oocyte yield in those cases undergoing oocyte retrieval shows close relationship between circulating AMH and oocyte yield ($r^2 = 0.36$, $p < 0.0001$) is shown in Figure 2.

Insulin-resistant PCOS

Table III summarizes an examination of ovarian responses to FSH and also circulating AMH concentrations in insulin-resistant women with PCOS ($n = 28$), compared with age-matched patients with normal insulin sensitivity ($n = 190$). It is clear that the most distinguishing feature between the groups is the FolsS8, representing the number of follicles undergoing recruitment at the beginning of stimulation. Interestingly, the number of follicles recruited subsequently per day (Fols/d) showed no difference from controls and showed values similar to those of the 30-year group in the analysis of the influence of age. This suggests that there was a large stockpile of follicles with intrinsic FSH sensitivity awaiting recruitment at the start of treatment but that the number of follicles subsequently progressing into the FSH-sensitive zone, and providing the figures for Fols/d, was effectively no different from age-matched controls.

Table III. Analyses of the responses of the IR-PCOS group compared with age-matched insulin-sensitive controls

		Mean	Lower 95% CI	Upper 95% CI	P-value
FolsS8	IR-PCOS	5.893	4.08	7.71	0.005
	InSen	3.311	2.86	3.77	
Fols/d	IR-PCOS	2.336	1.53	3.14	0.96
	InSen	2.195	1.95	2.44	
Oocytes	IR-PCOS	13.08	8.56	17.6	0.17
	InSen	9.211	8.26	10.2	
AMH	IR-PCOS	26.1	15.4	36.8	0.016
	InSen	13.34	11.6	15.1	

AMH, anti-Mullerian hormone; InSen, insulin sensitive; IR-PCOS, insulin-resistant polycystic ovary syndrome.

The oocyte yield was in the range expected from 25-year-old women (Table I), as was the FolsS8 value.

The significantly increased concentrations of AMH in the IR-PCOS group probably represents the increased cohort size in patients with PCOS, many of which are stockpiled in the FSH-sensitive zone. It exceeds the value observed in the 25-year-old group recorded above.

Discussion

We have examined the dynamics of observable follicular growth in response to excess circulating FSH in the controlled environment of ART cycles to explore the interrelationships between the categories of follicles and factors known to influence outcome of assisted conception. These include age, insulin resistance, the roles of the two putative circulating biochemical markers – FSH and AMH, and the practical aspect of oocyte yield. Data from the model described by Faddy (2000) has allowed us to relate the observations to the generic biology of follicular life, and the results show a number of interesting features in normal and exceptional circumstances, as in the IR-PCOS paradigm.

Increasing age was associated with an increasing confluence of the number of follicles attaining FSH sensitivity on a daily basis and the depletion rate of the PFP. The observations indicate that there was a negligible influence of age upon the initial cohort size responding to the FSH injections. This suggests that follicles can remain sensitive to FSH for a timeframe of days awaiting FSH stimulation (during down-regulation or in the normal ovarian cycle), leading to a stockpile of follicles whose dimensions are relatively independent of age. The only factor promoting the size of this stockpile was associated with insulin resistance in the IR-PCOS group.

The principle impact of age was seen on the number of follicles being recruited subsequent to the first cohort (the Fols/d value or the replacement rate). This may go some way to explain why increasing the dose of FSH during a treatment cycle in older women has little impact upon the oocyte yield (van Hooff *et al.*, 1993; Out *et al.*, 2000), as the follicles are simply not entering the FSH-sensitive zone at a high enough frequency.

The concepts developed here, of a pool of follicles recruitable immediately by FSH, and a secondary cohort constituted

from follicles attaining FSH sensitivity on a daily basis suggest that using the long-course protocol would deny the possibility of mild protocols aimed at lower stress and reduced oocyte yields. These may only be reliably attained with adaptation of the normal cycle where atresia is maintained by normal control mechanisms (Macklon and Fauser, 2003).

The data indicate that in younger women there remains a spare capacity of follicles (oocytes), to allow for either selection or wastage at the very earliest stages of development, but at ages 35 years and beyond nearly all follicles leaving the primordial pool proceed though to attain the ability to be recruited by FSH. This indicates that at these later ages, most of the follicular atresia occurs at the final stages of development, due to FSH deprivation, in contrast to younger women, where most follicular atresia takes place at the earliest stages of development (primordial-to-primary transition). This observation may relate to the increase in embryo aneuploidy rate with advancing age, as the confluence of PFP depletion and Fols/d reflects the loss of a potential locus of oocyte selection. The concepts of follicular dynamics were reviewed expertly by Gougeon (1996), and the data presented here add specific numbers to some of the daily events taking place.

If the ovarian reserve is defined as the sum of follicles remaining within the ovary, most of which are primordial, then it appears to have little relationship with the number of follicles able to attain FSH sensitivity. Correspondingly, it is the degree of atresia at the primordial-to-primary transition that is most profoundly influenced by age.

The data support previous indications that AMH is a reliable marker of the number of developed follicles attaining FSH sensitivity (Seifer *et al.*, 2002; van Rooij *et al.*, 2002; Laven *et al.*, 2004). To be accurate, this is not the same as the ovarian reserve which includes the large number of primordial follicles and which does not appear to contribute to the circulating AMH concentration (Weenen *et al.*, 2004). The data suggest that AMH in the circulation derives from both categories of follicles although the strongest correlation was with Fols/d. AMH is a strong predictor of the number of viable antral follicles and oocyte yield in ART cycles stimulated with FSH.

AMH may become the biochemical marker of greatest use in the IVF setting, but also in other areas of reproduction and health, such as through puberty and the impending menopause for which it may become a marker with greater precision than anything else available. More work should be aimed at establishing its roles and limitations in reproductive medicine.

The influence of insulin resistance in the follicular dynamics of PCOS was explored by comparison with the insulin-sensitive group and revealed that the main significant factor was the stockpile of FSH-sensitive follicles represented by FolsS8. This concurs with the report of Maciel *et al.* (2004) who described this stockpile effect in detail, and it is probably responsible for the raised circulating AMH concentrations. It is notable that the number of follicles attaining FSH sensitivity on a daily basis (Fols/d) was not different from controls. This suggests that in women with IR-PCOS, antral follicles maintain their FSH sensitivity longer than controls before proceeding to atresia, leading to an enlarged stockpile and excessive

responses to exogenous FSH. The previous observation that protracted treatment of women with PCOS with metformin results in a reduction of AMH (Fleming *et al.*, 2005; Piltonen *et al.*, 2005) indicates that the reduced exposure to insulin intrinsic in metformin treatment eventually results in a decline in the stockpile of FSH-sensitive follicles (represented by FolsS8). The observation that the principle difference in IR-PCOS is not the number of follicles attaining FSH sensitivity per day but the number of follicles maintaining FSH sensitivity may be potentially exploited in women who respond poorly to conventional stimulation in assisted reproduction (poor responders). The maintenance or promotion of FSH sensitivity in these cases may be a direct or indirect response to insulin or androgen, and it suggests that a clinical intervention to increase the size of this follicular pool may be possible. It is possible that a relatively short-term increase in insulin or androgen exposure may allow this to proceed. However, this effect may be limited as the strongest correlation with oocyte yield was the Fols/d value, not the FolsS8.

Overweight and obese women appeared to demonstrate follicular development profiles very similar to normal weight women, and oocyte yields and AMH were also unaffected by BMI within the range examined. The reduced fecundity described for women with a BMI > 27 kg/m² (Lintsen *et al.*, 2005) must derive from sources other than follicular dynamics.

In summary, these data reveal that the two cohorts of follicles observed in standard cycles of ART represent phenomena under different control mechanisms. Age appears to have no impact upon the initial response cohort, while insulin resistance promotes the size of this cohort. In contrast, the secondary cohort representing the follicles attaining FSH sensitivity on a daily basis is negatively influenced by age but unaffected by insulin resistance. Circulating AMH shows a strong correlation with both these follicle cohorts and is strongly predictive of oocyte yield. As a biochemical marker of ovarian activity, AMH may provide most information in the widest clinical circumstances.

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