Feed restriction in cyclic gilts: gonadotrophin-independent effects on follicular growth

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Feed restriction in cyclic gilts: Gonadotrophin-independent effects on follicular growth

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Abstract — Our objective was to determine whether changes in metabolic hormones, induced by feed restriction, can alter follicle distribution in swine ovaries through effects independent of LH pulsatility. In a factorial arrangement, 24 gilts were fed a high or a low level of dietary energy (240 or 80% of maintenance requirements) and given an antagonist of GnRH or saline between days 3 and 12 of the oestrous cycle. Serial blood samples were collected on day 12 and ovaries on day 13. Antagonist treatment, that blocked LH pulsatility, decreased the number of follicles larger than 2 mm and increased the number of follicles smaller than 1 mm. The feed restriction did not alter gonadotrophin secretion, decreased the number of follicles smaller than 1 mm and increased the number of 1- to 1.9-mm follicles. These findings indicate that feed restriction can alter the growth of small follicles independently of gonadotrophin levels.

gilt / food restriction / oestrous cycle / gonadotrophin / ovarian development

Résumé — Rationnement alimentaire chez la cochette cyclique : effets sur la croissance folliculaire ovarienne, indépendants des hormones gonadotropes. L'objectif de l'étude est de déterminer si l'altération des niveaux circulants des hormones impliquées dans le métabolisme des nutriments, induite par un rationnement alimentaire, peut modifier la distribution des follicules dans les ovaires de truies cycliques, par des effets indépendants des hormones gonadotropes. Du troisième au 12^{e} jour du cycle sexuel (J0 = début de l'œstrus), 24 truies reçoivent un niveau d'alimentation haut ou bas (240 ou 80 % de leurs besoins énergétiques d'entretien) et un traitement par un antagoniste du GnRH ou par une solution saline, selon un schéma factoriel. Des prélèvements sériés de sang sont réalisés à J12 et les ovaires sont prélevés à J13. Le traitement par l'antagoniste du GnRH, qui bloque la pulsatilité de LH, diminue le nombre de follicules > 2 mm et augmente le nombre de follicules < 1 mm. Le rationnement alimentaire n'a pas d'effet significatif sur la sécrétion des hormones gonadotropes, diminue le nombre de follicules < 1 mm et augmente le nombre de follicules

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de 1 à 1,9 mm. Ces résultats montrent que le rationnement alimentaire peut altérer la croissance des follicules indépendamment d'effets sur les hormones gonadotropes.

truie / cycle sexuel / rationnement alimentaire / hormones gonadotropes / développement ovarien

1. INTRODUCTION

There is increasing evidence that the effects of nutrition on ovarian function are not only mediated by an alteration of gonadotrophin secretion, but also by hormones and metabolites involved in nutrient metabolism acting directly on the ovarian level [3, 23]. In cyclic gilts, flushing increases ovulation rate [2, 8] and, in underfed prepubertal gilts, refeeding stimulates follicular development [7], possibly without altering plasma gonadotrophins. In reproductive sows, a reduced feed supply during lactation delays ovarian development and oestrus after weaning, through inhibition of LH secretion [28, 31], and increases the number of small follicles (< 1 mm) [28], whose growth may be independent of gonadotrophin levels. Indeed, porcine follicular growth has been divided into three periods: (1) independent of gonadotrophin action up to 2 mm in diameter, (2) dependent on FSH from 2 to 4 mm and (3) dependent on LH pulses beyond 4 mm [9]. Thus, we hypothesise that, in underfed sows, altered growth of follicles below 4 mm is mainly due to the effect of metabolic mediators directly at the ovarian level, whereas altered growth beyond 4 mm is primarily due to the inhibition of LH pulsatility (indirect effect).

Our purpose is to determine whether alterations in metabolic hormones, due to a feed restriction, can modify follicle distribution in swine ovaries, independently of changes in LH secretion. To achieve this, the effects of feed restriction were evaluated in gilts treated or not with an antagonist of GnRH. This treatment has been demonstrated to suppress LH pulsatility without an effect on FSH, and to allow follicular growth up to 4 mm in diameter [4, 9].

2. MATERIALS AND METHODS

2.1. Animals and experimental design

Twenty-four crossbred Landrace × Large White gilts, which averaged 219 ± 8 days of age (mean ± SD), had their third or fourth oestrous cycles synchronised by daily feeding of 20 mg·d^{-1} of altrenogest (Regumate®, Roussel-Uclaf, Romainville, France) for 18 days. They were checked for oestrus twice daily in the presence of a mature boar. The first day of oestrous behaviour was designated as day 0.

During the whole experiment, gilts received twice daily, at 08.30 and 13.40 h, a diet containing 3.04 Mcal of ME·kg^{-1}, 18.1% crude protein and 0.96% lysine (Tab. I). From the beginning of the altrenogest treatment until day 2, feed allowance for all gilts was 240% of the energy requirements for maintenance. Maintenance requirements were calculated for individual gilts on the basis of their metabolic body weight (0.24 Mcal ME·kg^{-1} of body weight$^{0.60}$ [20]). At day 2, which corresponded to the beginning of the luteal phase, gilts averaged 158 ± 14 kg body weight (mean ± SD) and were assigned to one of four treatments in a 2 × 2 factorial combination. From days 3 to 12, feed allowance was 240% and 80% of the energy requirements for maintenance for well-fed gilts (H) and restricted gilts (L), respectively. These levels of feeding represent approximately 90% and 30% of the voluntary feed intake, respectively. In the

Table I. Composition of the diet.

Ingredient, % (air-dried basis)	
Barley	24.00
Wheat	24.38
Yellow corn	15.00
Soybean meal	23.00
Wheat bran	5.00
Beat molasses	3.00
Fat	2.00
Calcium carbonate	1.40
Dicalcium phosphate	1.20
Salt	0.45
Trace mineral and vitamins[a]	0.50
L-Lysine HCl (78 %)	0.07
Analysed levels (as fed)	
Dry matter, %	88.8
Organic matter, %	58.5
Crude protein, %	18.1
Digestible energy, Mcal·kg^{-1}	3.2
Amino acids content[b]	
Lysine, %	0.96
Methionine + cystine, %	0.62
Threonine, %	0.65

[a] Provided the following amounts of trace elements in milligrams per kilogram: 80 mg of iron; 10 mg of copper; 40 mg of manganese; 100 mg of zinc; 0.1 mg of cobalt; 0.2 mg of iodine; 0.15 mg of selenium; and vitamins in units or milligrams per day: vitamin A, 5 000 IU; vitamin D3, 1 000 IU; vitamin E, 20 mg; vitamin K3, 2 mg; thiamin, 2 mg; riboflavin, 4 mg; nicotinic acid, 15 mg; d-pantothenic acid, 10 mg; pyridoxin, 1 mg; d-biotin: 0.2 mg; folic acid, 1 mg; vitamin B12, 0.02 mg; choline: 500 mg.
[b] Calculated values (INRA, 1989).

L group, there was no feed refusals. In the H group, feed refusals were rarely observed and were not measured. During this period, restricted gilts lost live weight whereas well-fed females gained weight (–2.4 ± 0.9 vs. +12.9 ± 0.8 kg, mean ± SD, $P < 0.001$). From days 3 to 12, gilts received either 0.6 mg of Antarelix (A, $n = 12$) or saline as a sham treatment (C, $n = 12$) twice daily (at 09.00 and 21.00 h). All gilts were slaughtered on day 13, i.e. in the late luteal phase, between 08.00 and 09.00 h. This timing of treatments (days 3–12) was selected in order to assure ovulation of all gilts before starting gonadotrophin deprivation, to apply a feed restriction long enough to induce marked alterations in metabolic hormones and to end before luteolysis.

2.2. Sample collection

Jugular vein catheterisation was performed on the 24 gilts under general anaesthesia during the last week of altrenogest administration. Sows were deprived of feed on the day of surgery and then refed. Catheters were rinsed daily with physiological serum containing sodium heparin (190 IU·mL^{-1}) and an antibiotic. Blood samples (5 mL) were collected every 15 min from 10.00 to 16.00 h on day 12. Samples were collected in heparinised tubes kept on ice and centrifuged for removal of plasma. Plasma samples were stored at –20 °C until assayed.

At slaughter, ovaries were collected within 15 min after death and weighed. All corpora lutea were counted. The diameter of the largest follicle was determined using a calliper rule. Ovaries were frozen in liquid nitrogen after coating with a cryoprotectant embedding medium (Tissue-Tek, Miles Inc., Elkhart, IN, USA) (left ovaries) or not (right ovaries). They were stored at –70 °C until histology (left ovaries) or membrane preparation (right ovaries [15]).

2.3. Hormone assays

Plasma LH concentrations were measured every 15 min, FSH concentrations every hour and progesterone once daily (at 16.00 h). They were determined by validated RIAs (LH and FSH [6]; progesterone [30]). Samples were analysed in duplicate within a single assay. Assay sensitivities, estimated as 90% of total binding, were 0.8 ng·mL^{-1} for LH, 1 ng·mL^{-1} for FSH and 3.3 ng·mL^{-1} for progesterone. The intraassay CV was 7.5% at 2.55 ng·mL^{-1} for LH,

2.7% at 2.00 ng·mL^{-1} for FSH and 4.1% at 28.2 ng·mL^{-1} for progesterone. The interassay CV was 15.9% at 1.51 ng·mL^{-1} for LH, 11.2% at 2.24 ng·mL^{-1} for FSH and 15% at 48.7 ng·mL^{-1} for progesterone.

2.4. Histological procedures

Left ovaries were serially sectioned at 10 µm with an ultramicrotome maintained at –23 °C, as previously described [28]. One out of 10 sections was mounted, fixed, stained with Feulgen and observed microscopically. All the antral follicles (measuring at least ~0.4 mm) were counted, measured and classified as healthy or atretic. A follicle was classified as atretic when five or more pyknotic bodies were counted among the granulosa cells of the section studied. Counting was done on the section where the oocyte was found. Antral follicles were grouped into four classes according to their diameter: class 1: < 1 mm; class 2: 1–1.9 mm; class 3: 2–4 mm; class 4: > 4 mm.

2.5. Statistical analyses

Profiles of LH were analysed as previously described [24]. Mean concentrations of FSH and characteristics of LH secretion (mean and basal concentrations, number of pulses) were calculated and used for statistical analyses.

All data (numbers and percentages of follicles and hormonal levels) were analysed by analysis of variance using the GLM procedure of SAS [29]. All models included the effect of feeding level, Antarelix treatment, and the interaction between these two factors. The analysis of the number of follicles within specific size class was performed with the total number of follicles in the ovary introduced as a covariate. For progesterone analysis, the number of corpora lutea was introduced as a covariate. Percentages of atresia were analysed after arc sin $\sqrt{}$ transformation.

3. RESULTS

3.1. Gonadotrophic hormones and progesterone

No interaction between the level of feeding and Antarelix treatment was found. The level of feeding had no influence on the characteristics of LH secretion (Tab. II). Antarelix treatment had no effect on basal

Table II. Influence of the level of feeding and Antarelix treatment on characteristics of LH profiles and mean concentrations of FSH (mean ± SEM). Control gilts: well-fed (CH) or feed-restricted (CL); Antarelix-treated gilts: well-fed (AH) or feed-restricted (AL).

	(n = 6)				Sign.[a]	
	CH	CL	AH	AL	FL[b]	A[c]
Mean LH, ng·mL^{-1}	1.17 ± 0.06	1.15 ± 0.03	1.07 ± 0.04	1.01 ± 0.01	NS	**
Basal LH, ng·mL^{-1}	1.03 ± 0.48	1.02 ± 0.01	1.07 ± 0.04	1.01 ± 0.01	NS	NS
LH pulses (for 6 h)	1.17 ± 0.16^{x}	1.17 ± 0.17^{x}	0 ± 0^{y}	0 ± 0^{y}	NS	***
Mean FSH, ng·mL^{-1}	1.64 ± 0.06	1.83 ± 0.15	1.67 ± 0.14	1.73 ± 0.09	NS	NS

[a] Statistical significance. NS: $P > 0.10$; ** $P < 0.01$; *** $P < 0.001$.
[b] Influence of feeding level (FL).
[c] Influence of Antarelix treatment (A).
No interaction between feeding level and Antarelix treatment was found.
[x, y] Means within a row lacking a common superscript letter differ ($P < 0.05$).

LH ($P > 0.10$), but reduced mean LH concentration ($P < 0.01$) and the number of LH pulses ($P < 0.001$, Tab. II). The level of FSH was not influenced by the level of feeding nor by Antarelix treatment (Tab. II). Mean progesterone concentration was significantly reduced by Antarelix treatment ($P < 0.01$; Fig. 1) and tended to be increased by feed restriction ($P = 0.05$). When the number of corpora lutea was introduced as a covariate in the analysis, the effect of feed restriction was not significant ($P = 0.098$).

3.2. Macroscopic characteristics of the ovaries

The number of corpora lutea counted in the ovaries on day 13 of the oestrous cycle was similar for the four groups of gilts (Tab. III). The weight of the ovaries and the maximal follicular diameter were significantly reduced by Antarelix treatment (Tab. III). The weight of the ovaries was also reduced by feed restriction ($P < 0.05$, Tab. III).

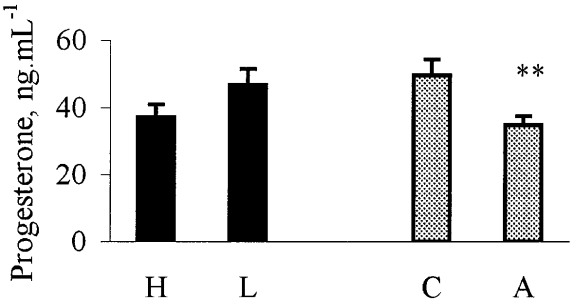

Figure 1. Influence of the level of feeding (H vs. L) and Antarelix treatment (C vs. A) on mean plasma concentrations of progesterone on day 12 of the oestrous cycle in gilts (mean ± SEM; $n = 12$ group^{-1}). ** $P < 0.01$.

Table III. Influence of the level of feeding and Antarelix treatment on macroscopic characteristics of both ovaries of gilts (mean ± SEM). Control gilts: well-fed (CH) or feed-restricted (CL); Antarelix-treated gilts: well-fed (AH) or feed-restricted (AL).

	($n = 6$)				Sign.[a]	
	CH	CL	AH	AL	FL[b]	A[c]
Number of corpora lutea per gilt	18.5 ± 1.5	18.0 ± 1.1	18.5 ± 0.8	20.2 ± 1.4	NS	NS
Ovarian weight, g	20.4 ± 1.6^{x}	15.8 ± 1.0^{xy}	13.8 ± 1.3^{y}	12.2 ± 0.7^{y}	*	***
Maximum follicular diameter, mm[d]	4.6 ± 0.3^{x}	4.2 ± 0.1^{x}	3.1 ± 0.3^{y}	3.2 ± 0.2^{y}	NS	***

[a] Statistical significance. NS: $P > 0.10$; * $P < 0.05$; *** $P < 0.001$.
[b] Influence of feeding level (FL).
[c] Influence of Antarelix treatment (A).
[d] Diameter of the largest follicle per animal, taking both ovaries into account.
No interaction between feeding level and Antarelix treatment was found.

3.3. Ovarian populations of antral follicles

No interaction between the level of feeding and Antarelix treatment was found for the total number of follicles. Neither the level of feeding nor Antarelix treatment significantly altered the number of healthy antral follicles (95 ± 40, mean ± SD) nor the number of total (healthy + atretic) antral follicles (148 ± 55, mean ± SD) in the left ovaries ($P > 0.1$).

The interaction between the level of feeding and Antarelix treatment was not significant for the numbers of healthy, atretic or total follicles in the various size classes (Tab. IV). Feed-restricted gilts exhibited a lower number of follicles in class 1 (healthy, $P < 0.01$ and total, $P < 0.05$) and a higher number of healthy and total follicles in class 2 ($P < 0.05$; Fig. 2) than well-fed gilts. Antarelix-treated gilts exhibited a higher number of healthy and total follicles in class 1 ($P < 0.05$), a lower number of follicles (healthy, atretic and total) in class 3 ($P < 0.05$) and much fewer follicles > 4 mm ($P < 0.001$; Fig. 2) than control gilts.

Whatever the size class, the treatments did not significantly alter the percentage of atresia (8.9% in class 1; 59.0% in class 2; 72.1% in class 3 and 30.6% in class 4; $P > 0.10$).

4. DISCUSSION

Treatment with the GnRH antagonist Antarelix completely blocked the pulsatility of LH without affecting basal LH and FSH secretion, as previously described [9]. Although Antarelix-treated gilts exhibited fewer follicles larger than 2 mm in diameter than control gilts, and thus presumably less plasma inhibin, their levels of FSH were similar. This suggests that the potential negative effect of Antarelix on FSH release from pituitary stores was counterbalanced by the lower negative feedback exerted by the ovaries.

Ovaries from Antarelix-treated gilts contained no or few follicles larger than 4 mm, in agreement with previous data [4, 9]. Antarelix treatment also decreased the number of 2- to 4-mm follicles and increased the number of follicles smaller than 1 mm. Driancourt et al. [9] reported no significant effect of Antarelix on these follicles. Such a difference may be related to technical differences (limits of size classes or use of fixed vs. frozen tissue) or to the treatment duration (10 days vs. 7 days). Our findings showed that LH pulses stimulate the growth of follicles larger than 1 mm, in agreement with the observation of LH receptors and messengers in theca cells of small antral follicles [14, 19]. The inhibition of LH pulses results in the accumulation of follicles smaller than 1 mm in the ovaries.

Table IV. Significance of the effects of the level of feeding (FL), Antarelix treatment (A) and the interaction FL × A on mean numbers of follicles in specific size class in the left ovaries of gilts.

	Healthy follicles			Atretic follicles			Total number		
Size class	FL	A	FL × A	FL	A	FL × A	FL	A	FL × A
< 1 mm	0.006	0.0002	0.325	0.783	0.874	0.673	0.011	0.0003	0.462
1–1.9 mm	0.015	0.085	0.952	0.814	0.076	0.241	0.016	0.692	0.190
2–4 mm	0.946	0.010	0.398	0.181	0.011	0.084	0.301	0.002	0.0842
> 4 mm	0.894	0.001	0.364	0.428	0.0001	0.807	0.518	0.0001	0.479

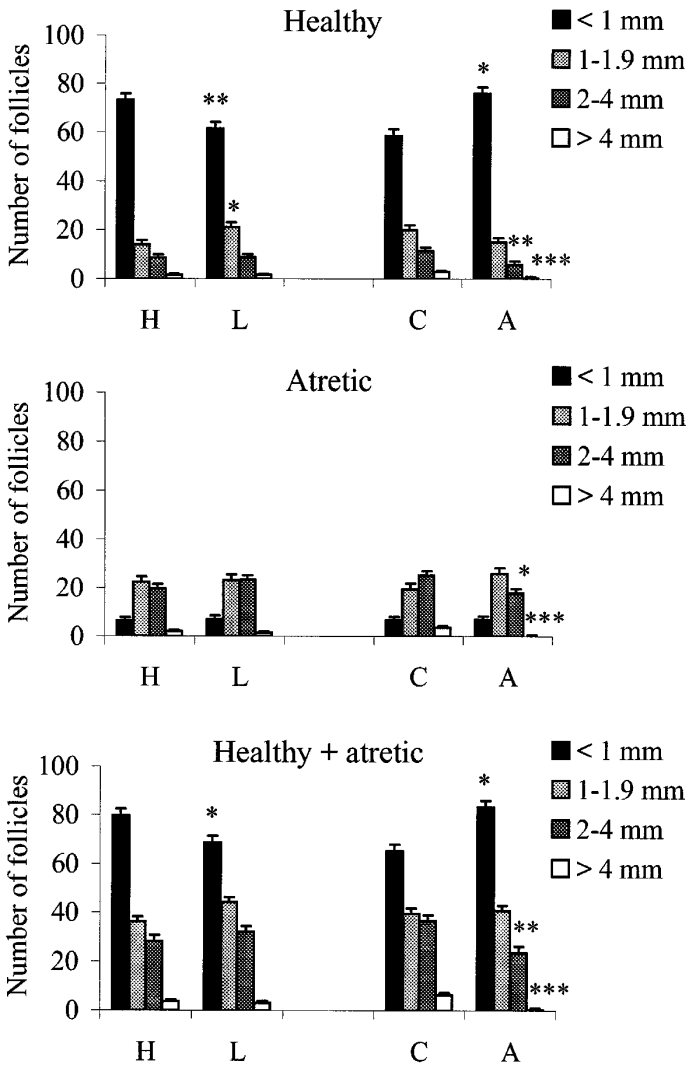

Figure 2. Influence of the level of feeding (H vs. L) and Antarelix treatment (C vs. A) on mean number of antral follicles in specific size classes on day 13 of the oestrous cycle in gilt left ovaries (adjusted mean ± SEM; n = 12 group^{-1}). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

As expected, the 10-day feed restriction stopped female growth and significantly reduced mean plasma concentrations of insulin, IGF-I and leptin (insulin: 44.4 ± 4.6 and 20.7 ± 1.5 μIU·mL^{-1}; IGF-I: 231.1 ± 8.2 and 161.7 ± 13.0 ng·mL^{-1}; leptin: 3.51 ± 0.2 and 2.24 ± 0.1 ng·mL^{-1} in H and L gilts, respectively; $P < 0.001$ [15]). It did not alter gonadotrophin secretion, in accordance with data from Flowers et al. [11] who noticed no effect of a 7-day feed restriction (~130% vs. ~270% of requirements for maintenance) on LH pulsatility in gilts during the late luteal phase. These findings indicate that low plasma insulin, IGF-I and leptin are not necessarily associated with reduced secretion

of gonadotrophins in cyclic gilts, whereas such associations have been observed in lactating sows [16, 21, 27].

The level of feeding had no influence on the number of follicles larger than 2 mm, but significantly altered the distribution of follicles smaller than 2 mm. Feed-restricted gilts exhibited less follicles in class 1 (< 1 mm) and more follicles in class 2 (1–1.9 mm) than well-fed gilts. This accumulation of 1–1.9 mm follicles suggests that, in feed-restricted gilts, more < 1 mm follicles grew to the larger class, whereas growth beyond 2 mm was reduced. This was observed in the presence or the absence of LH pulses and without changes in mean LH and FSH levels, supporting our hypothesis of a gonadotrophin-independent effect of feed restriction on follicular growth. The stimulated growth from class 1 to class 2 possibly involves growth hormone (GH) action. The feed restriction may have induced an increase in GH levels, as previously reported in growing pigs [5] and lactating sows [27] and this increase may have stimulated growth of very small follicles, which present abundant binding sites for GH [26].

We previously reported an increase in the percentage of healthy follicles smaller than 1 mm and a decrease in the percentage of healthy 1- to 3-mm follicles, related to the total number of follicles, in feed-restricted lactating sows [28]. In these females, LH pulsatility was strongly inhibited by feed restriction. In light of the present findings, it appears that the accumulation of follicles smaller than 1 mm in feed-restricted sows results more from the nutritionally-induced inhibition of LH pulsatility than from the direct influence of metabolic mediators at the ovarian level.

As mentioned above, the feed-restricted gilts (L group) exhibited reduced levels of insulin and IGF-I [15]. Insulin and IGF-I are known to amplify the stimulating action of gonadotrophins on follicular growth and maturation [1, 12] and to reduce follicular atresia in small (≤ 3 mm) and medium (4 to 6 mm) follicles from prepubertal gilts [17, 18]. Therefore, it seems surprising to find no effect on rate of atresia and no interaction between the level of feeding and Antarelix treatment. However, no effect of feed restriction on the rates of atresia was previously reported in prepuberal gilts [10] and in lactating sows [28]. Moreover, describing follicle distribution in size class does not fully characterise follicular populations, and we cannot exclude that the treatments induced biochemical alterations of follicles, such as changes in steroidogenesis or in IGF-I intrafollicular levels.

Finally, luteal activity was altered by Antarelix treatment, as indicated by the decrease in individual weight of the corpora lutea (data not shown) and progesterone level in Antarelix-treated compared with control gilts. Therefore, although late corpus luteum function did not require high LH support, LH plays a luteotrophic role, in agreement with the observation of LH receptors in functional corpora lutea [19, 32]. The level of feeding slightly altered progesterone levels, but not significantly. Previous findings have demonstrated higher progesterone level in feed-restricted sows, either at luteolysis [13] or during luteal development after ovulation [25], possibly due to a decreased metabolic clearance [22].

In summary, the main conclusion of this study is that feed restriction alters the growth of follicles smaller than 2 mm, independently of gonadotrophin levels.

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