

1 **Supplemental Material**

2 Supplemental Table 1: Donor dog source (breed and age, if known) and numbers of
 3 preantral, early antral, and antral stage follicles cultured in each experimental replicate.

Experimental Replicate	Dog Breed	Age (yrs)	Age Group	# Follicles per Stage		
				Preantral	Early Antral	Antral
1	N/A	4	Adult	2	6	6
2	N/A	1	Peri-pubertal	0	2	4
3	N/A	2	Adult	1	1	5
4	Labrador Retriever	0.8	Peri-pubertal	0	5	14
5	N/A	0.8	Peri-pubertal	4	7	12
6	Pooled culture: 2 Pomeranians (likely siblings)	0.5	Prepubertal	2	13	15
7	Chihuahua	0.8	Peri-pubertal	3	1	9
8	Labrador Mix	2	Adult	0	5	6
9	Poodle	0.8	Peri-pubertal	2	18	13
10	Chihuahua	3	Adult	1	11	4
11	German Shep. Mix	9	Aged	0	10	11
12	N/A	0.8	Peri-pubertal	0	15	22
13	Pooled culture: 2x Unk. Breeds	0.5, 1.5 yr	N/A	1	4	6
14	Cavalier King Charles	8.5	Aged	3	7	11
15	Pooled Culture: Mixed breed, Blue Heeler	both 1.5	Peri-pubertal	3	49	22
16	Pooled culture: 2 Pitt Bulls	N/A	N/A	15	38	6
17	Pooled culture: 2x Unk. Breeds	1, 4 yr	N/A	2	13	9
18	German Shepherd	0.9	Peri-pubertal	22	33	10
19	Pooled culture: 2x Unk. Breeds	0.4	Prepubertal	4	28	18
20	Pooled culture: 2 Unk.	both 0.8	Peri-pubertal	3	23	9

Breeds (likely siblings)						
21	Pooled culture: 2x Mixed Breeds	both ~ 1	Peri-pubertal	2	17	38
22	N/A	N/A	N/A	7	14	1
23	N/A	N/A	N/A	13	16	2
24	Pooled culture: Setter mix and Pug	both ~ 1	Peri-pubertal	6	4	3
25	Labrador Mix	0.8	Peri-pubertal	7	23	8
26	N/A	N/A		1	23	19
27	Labrador Mix	0.8	Peri-pubertal	5	18	11
28	Australian Blue Heeler	0.6	Prepubertal	1	9	11
29	Labrador	0.7	Peri-pubertal	4	6	9
30	Jack Russel Terrier	0.7	Peri-pubertal	2	15	5
31	Labrador Mix	0.7	Prepubertal	8	25	6
32	Jack Russel Terrier	3	Adult	8	11	7
33	N/A	N/A	N/A	4	10	7

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6 Supplemental Table 2: Numbers of dog follicles of each developmental stage cultured *in vitro*
 7 for 12 or 21 days across 48 treatment groups (activin, 0, 100, or 200 ng/ml; and FSH, 0, 0.1,
 8 1 or 10 µg/ml).

Treatment		Follicle Stage at Isolation		
Activin (ng/ml)	FSH (µg/ml)	Preantral	Early Antral	Antral
0	0	12	47	33
	0.1	6	29	29
	1	10	29	25
	10	20	52	28
100	0	8	57	31
	0.1	10	28	22
	1	11	29	23
	10	19	48	37
200	0	7	51	32
	0.1	10	24	22
	1	4	36	17
	10	19	50	40

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11 Supplemental Table 3: Numbers of dog follicles of each developmental stage cultured in vitro
 12 for 3 days 6 treatment groups (activin, 0, 100, or 200 ng/ml; and FSH, 0 or 10 µg/ml) for
 13 *FSHR* expression.

Treatment		Follicle Stage at Isolation		
Activin (ng/ml)	FSH (µg/ml)	Preantral	Early Antral	Antral
0	0	20	63	23
	10	30	60	20
100	0	17	56	33
	10	25	54	26
200	0	26	49	32
	10	24	58	23

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17 Supplemental Table 4: Primers utilized in qRT-PCR assessments of cultured follicles. qRT-
 18 PCR was run with final primer concentrations of 1 mM with 10 min preincubation at 95 C,
 19 followed by 55 cycles of amplification (30 sec at 95 C, 10 sec at annealing temperature, 10
 20 sec at 72 C).

Gene	Accession	Sequence	Annealing Temp (°C)	Primer Efficiency	Product Size (bp)
<i>β-Actin</i>	XM_845524.1	F: TCGCTGACAGGATGCAGAAG	60	0.819	127
		R: GTGGACAGTGAGGCCAGGAT			
<i>FSHR</i>	XM_014117520.1	F: ATTAGCATCCTGGCCATCAC	60	0.909	122
		R: CCAATGCAGAGATCAGCAAA			

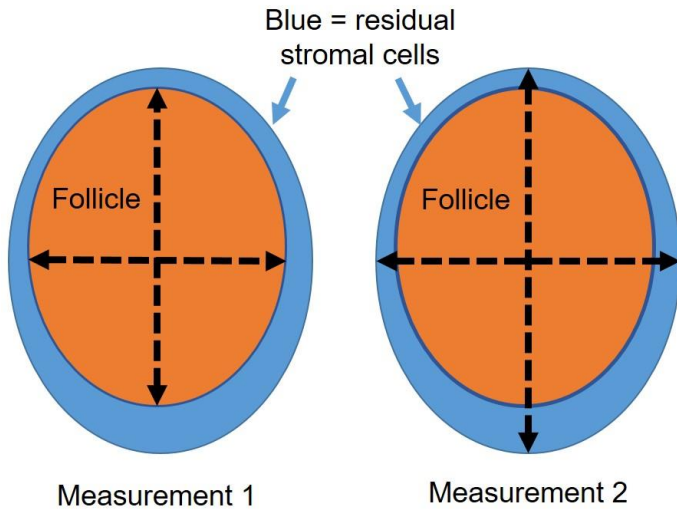
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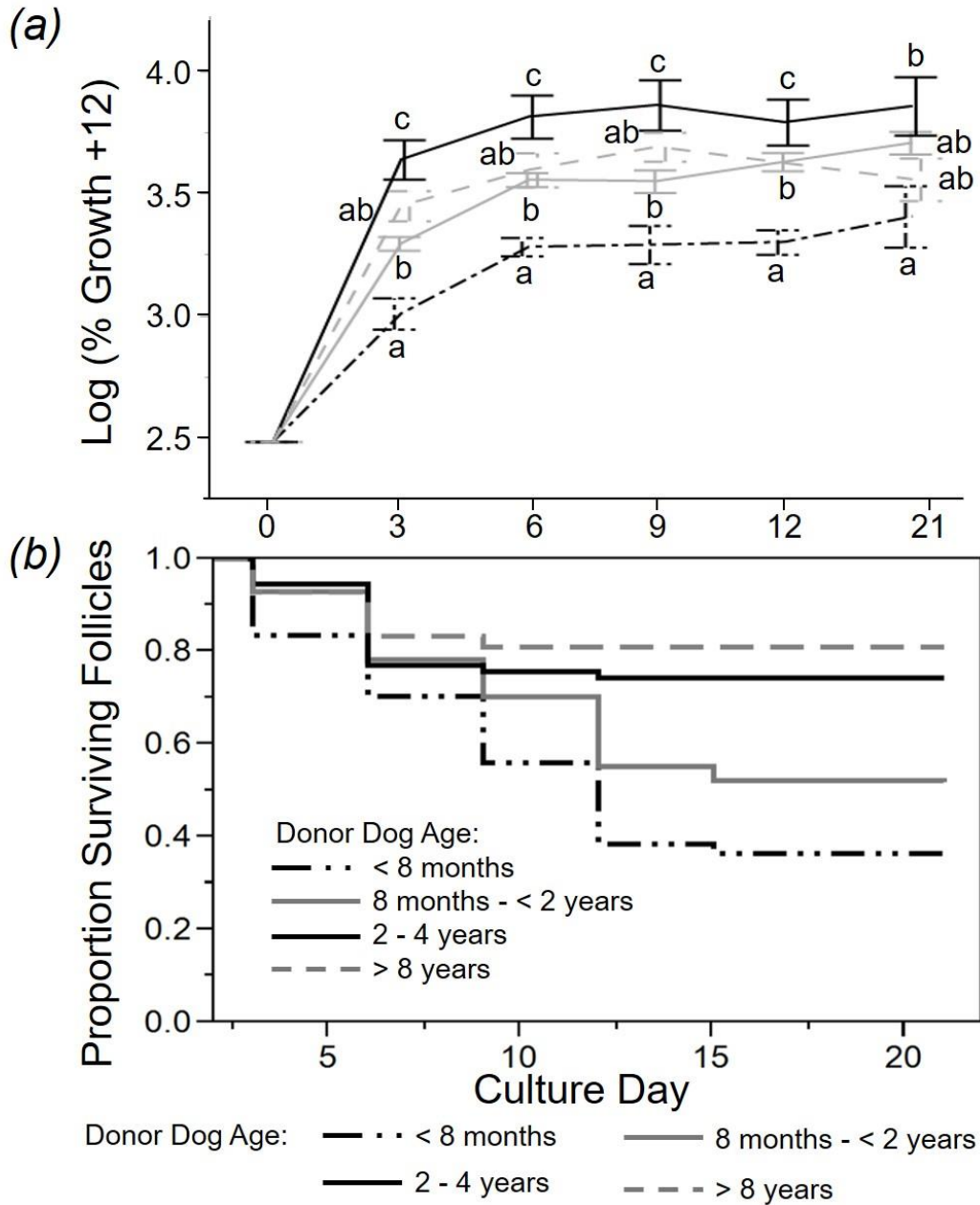
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27 Supplemental Figure 1: Diagram of follicle diameter measurements, with measurement #1
28 assessing the size of the follicle only, to determine follicle developmental stage at
29 isolation/onset of culture, and measurement #2 of the follicle plus residual somatic cells,
30 used in the evaluation of growth over the culture period.

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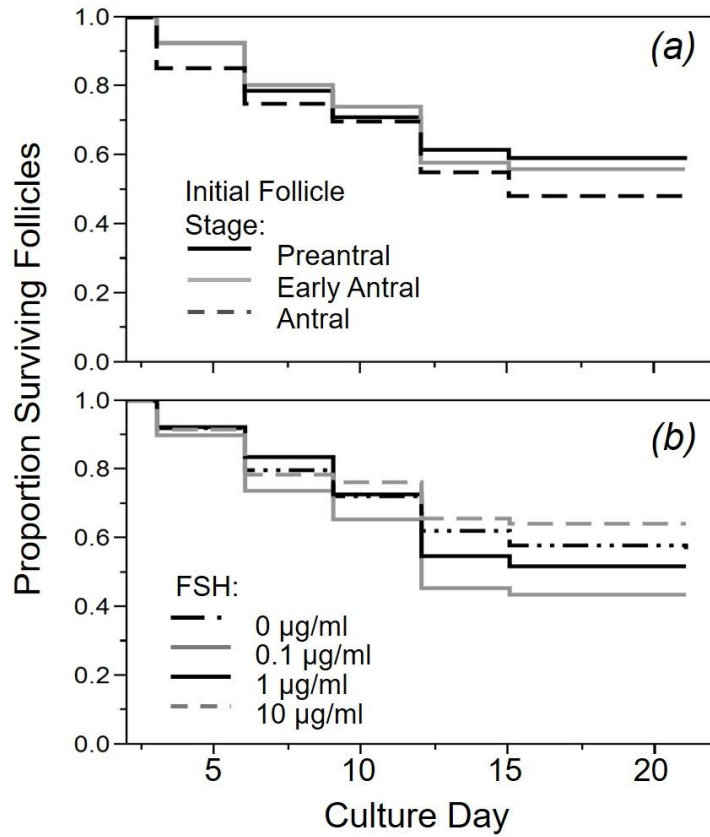


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35 Supplemental Figure 2: Dog ovarian follicle (A) growth and (B) Kaplan-meir survival plot by
 36 donor dog age grouped into categories of <8 mo (prepubertal), 8 mo to <2 y (young adult), 2
 37 to 4 y (prime breeding age), and >8 y (aged). Values with different letters on the same culture
 38 day are different ($P < 0.05$) among age groups.

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42 Supplemental Figure 3: Kaplan-meir survival plot of isolated dog ovarian follicles cultured *in*

43 *vitro* on the basis of (A) initial stage at incubation onset, and (B) supplementation with FSH at

44 0, 0.1, 1, or 10 µg/ml

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