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***Our publications related to this material can be accessed under Researcher Resources at [www.oncofertility.northwestern.edu](http://www.oncofertility.northwestern.edu).***

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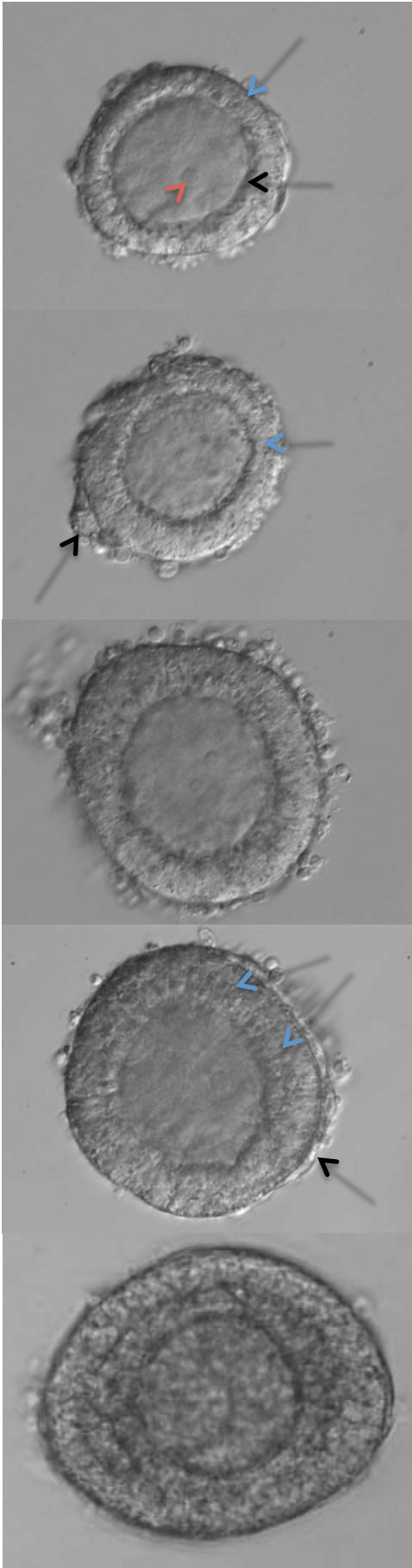
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# Follicle Reference Guide



Woodruff Laboratory  
Version 1.1 01/2010

## Classification and Anatomy of Follicles



-Primary follicle, one layer of cuboidal granulosa cells (GC) surrounded by the basement membrane (blue arrow). Size 85 $\mu$ m. The oocyte is the round, smooth disc in the center (black arrow). The small circle within the oocyte is the germinal vesicle (red arrow).

-Primary to secondary transitional follicle, one layer of GC (blue arrow), some stromal cells attached (black arrow). Size 85 $\mu$ m.

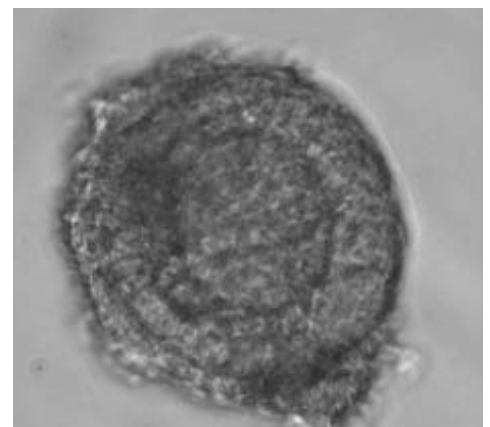
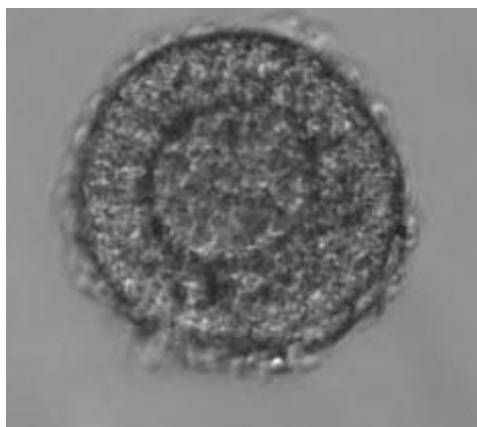
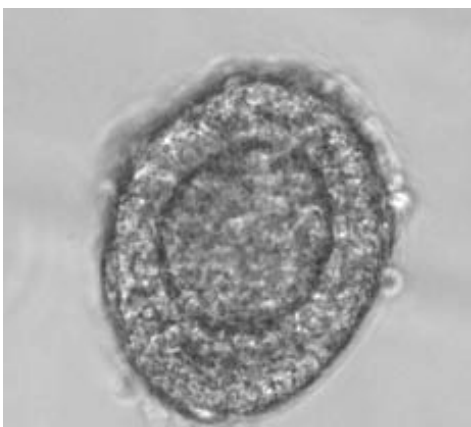
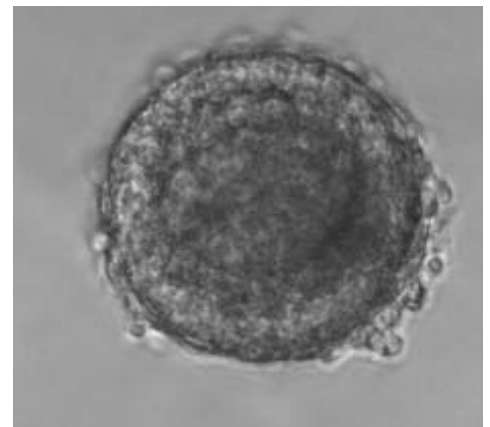
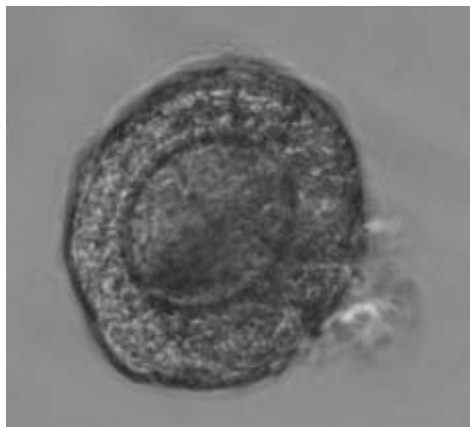
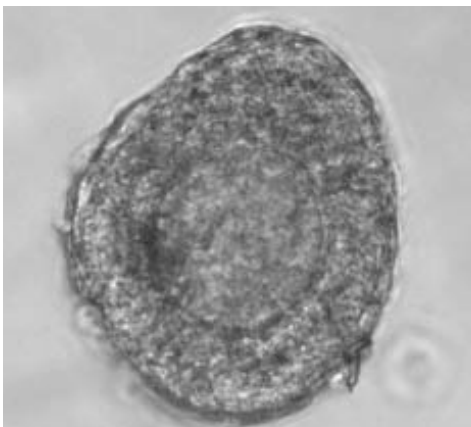
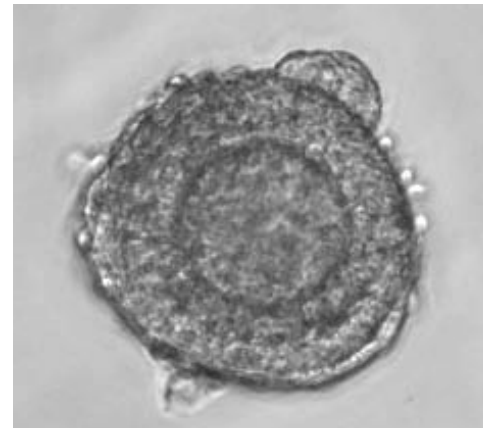
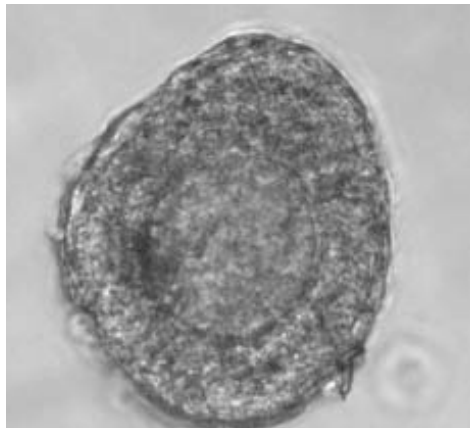
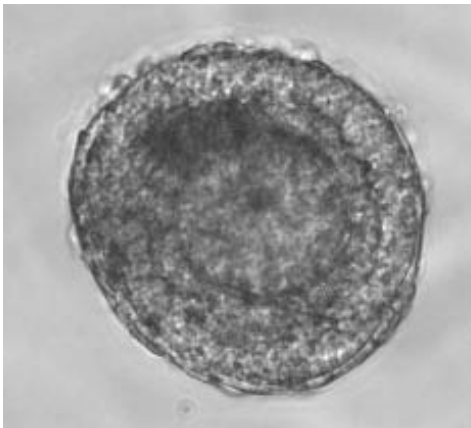
-Early secondary follicle, two complete layers of GC, however the borders among the GC are not distinguishable, some stromal cells attached. Size 114 $\mu$ m.

-Middle secondary follicle, complete two layers GC, the borders among GC can be observed (blue arrows), theca cells (flattened, disk shaped cells indicated by black arrow) can be found outside of basement membrane. Size 117 $\mu$ m

-Later secondary follicle, complete two layers GC, the borders among GC can be observed, TC can be found outside of basement membrane, some stroma cells attached, size 128 $\mu$ m

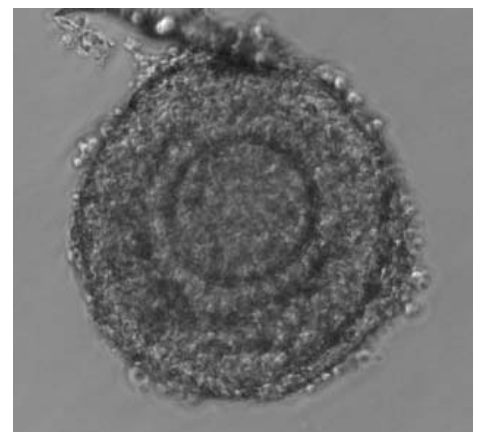
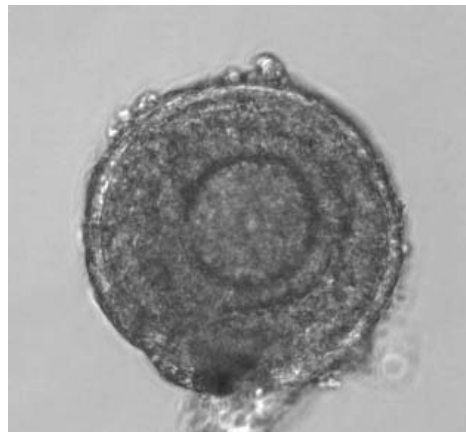
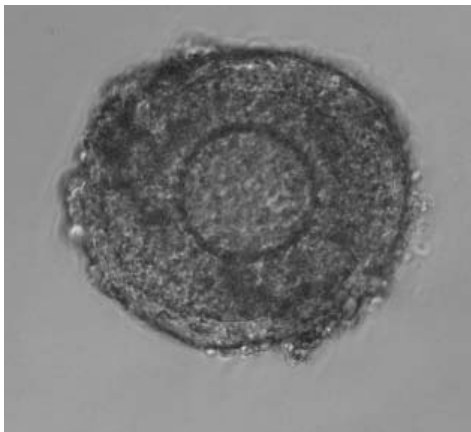
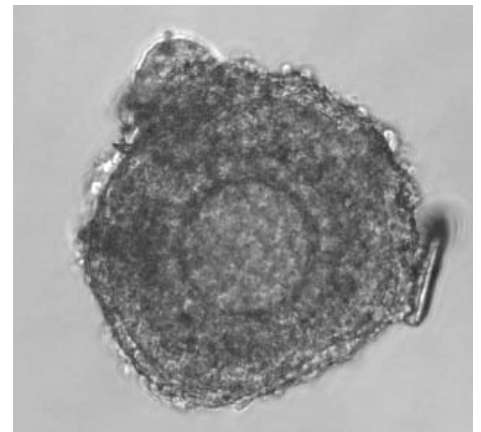
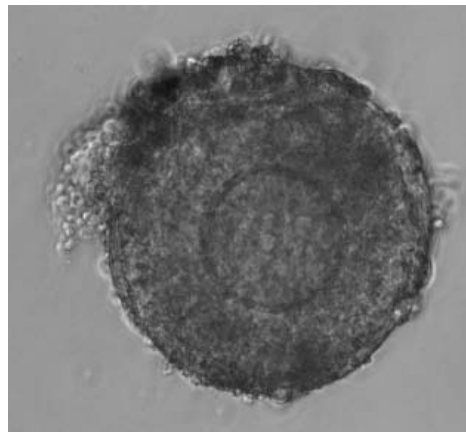
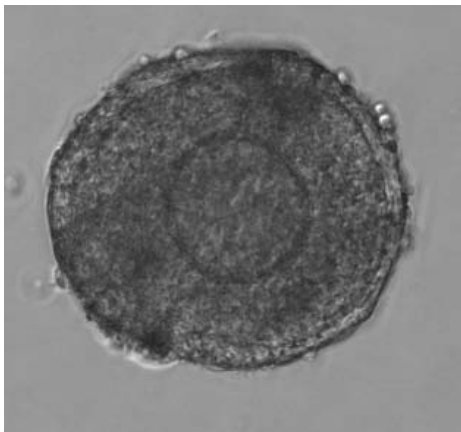
Healthy two-layer secondary follicles freshly isolated (culture day 0)  
from 12 day old mice.

A healthy follicle has a round, clearly defined, and undamaged basement membrane. The oocyte is visible and round and the germinal vesicle can usually be visualized without any difficulty. Damaged basement membranes or severely misshapen oocytes are indications of poor follicle quality and should not be selected for culture, as they will either not grow or will die. Additionally, do not select follicles that may be dark on the inside, even if they are round and within the proper range (120-150  $\mu\text{m}$  for two layer).



Healthy multi-layer secondary follicles freshly isolated (culture day 0) from 16 day old mice.

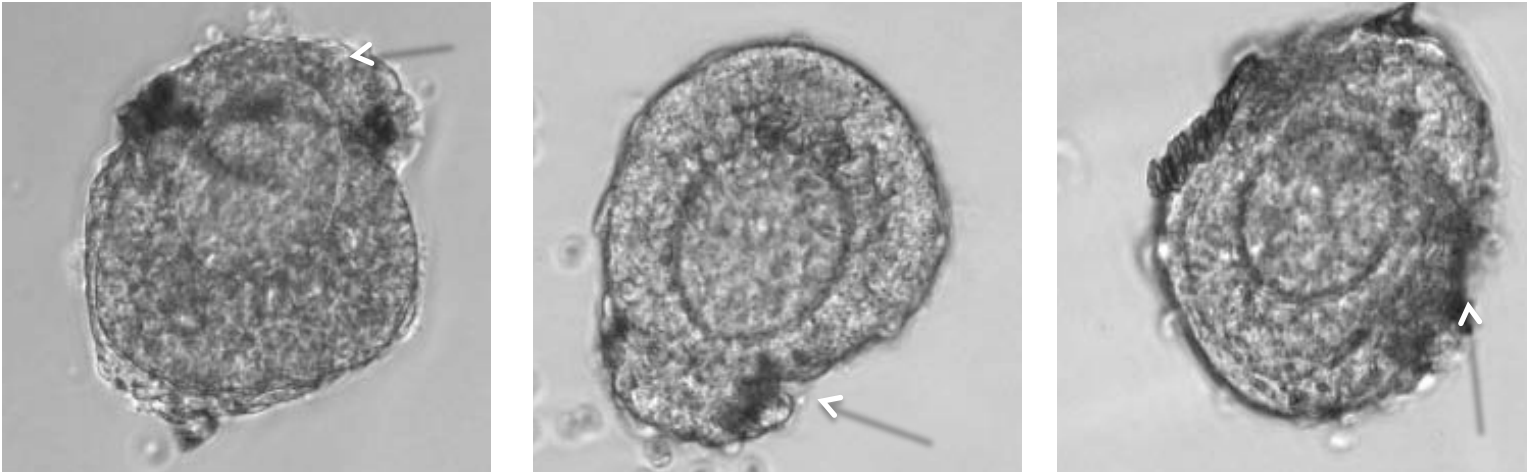
A multi-layer secondary follicle is characterized by more than two distinct layers of granulosa cells. It is important to note that granulosa cells themselves are all approximately the same size. Instead of expanding in size, granulosa cells proliferate and rearrange themselves to form a new layer within the basement membrane. Select healthy multi-layer secondary follicles using the same morphological quality criteria that you would use when selecting two layer secondary follicles: overall round shape, undamaged and defined basement membrane, clearly defined granulosa cells (not fuzzy, dark overall follicles. You will not necessarily see each granulosa cell membrane, but be able to see the basement membrane, layers of granulosa cells, and the oocyte), a round and undamaged oocyte. Ideal size range of multi-layer secondary follicles for good growth is between 150-180 $\mu$ m.



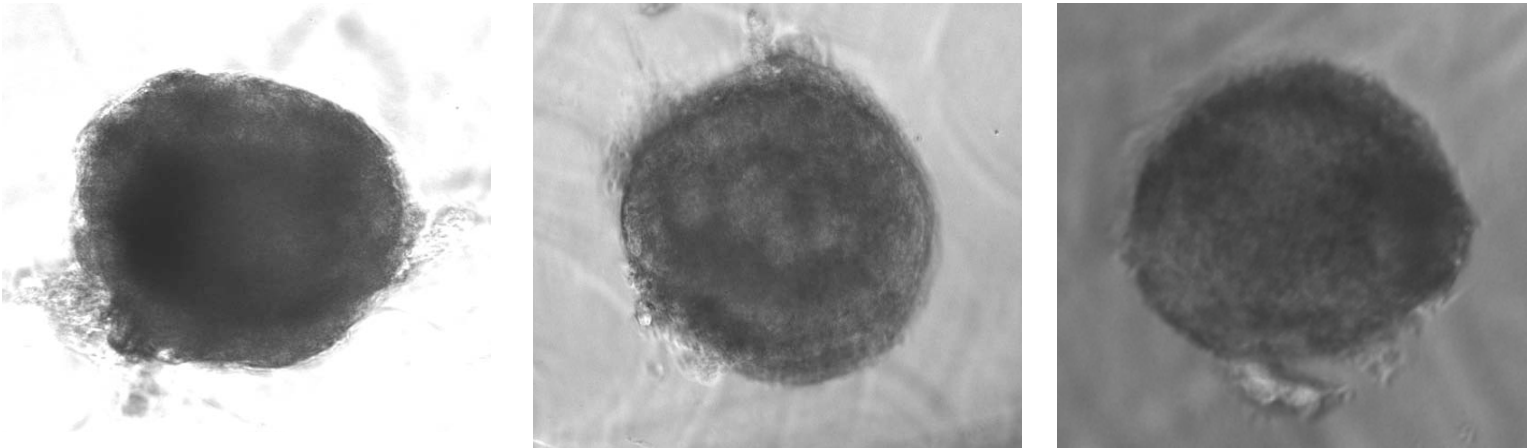
## Impaired Follicles

Avoid selecting severely oblong, misshapen follicles. Select only follicles with defined, undamaged basement membranes. Avoid dented, stretched, or gouged follicles, follicles that are overall dark or fuzzy, and follicles with oblong or dented oocytes or with a fuzzy border around the oocyte. Damaged or severely irregular follicles will not grow.

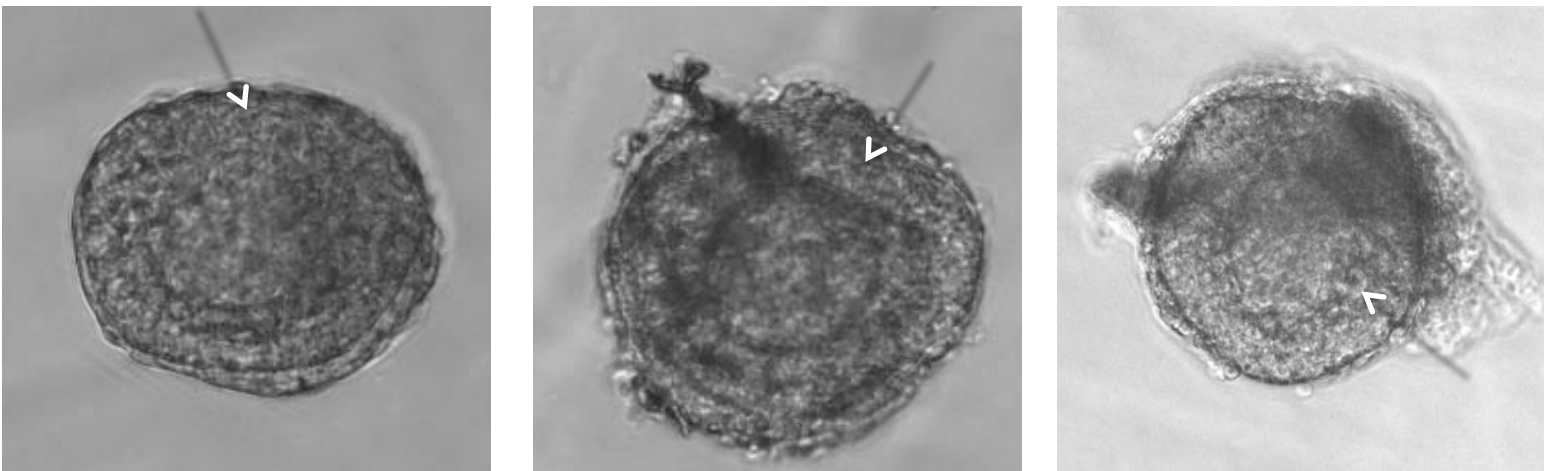
### Basement membrane ruptured



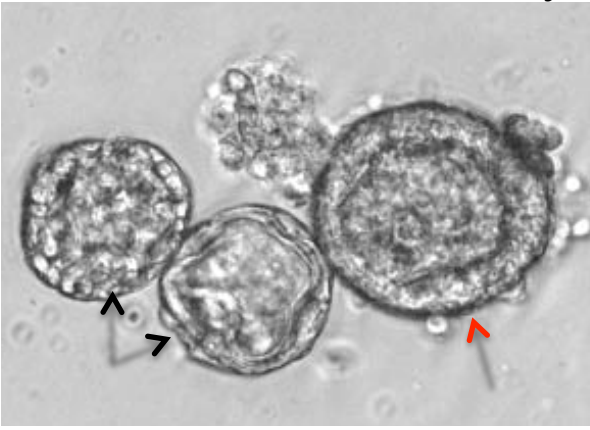
### Dark follicle with unclear basement membrane and/or oocyte



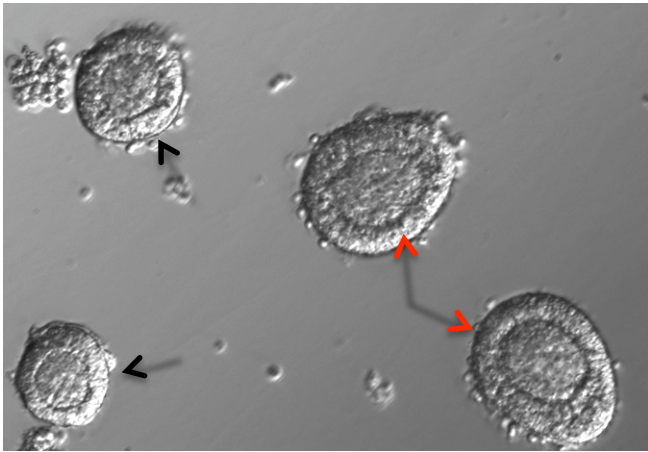
### Oocyte dented



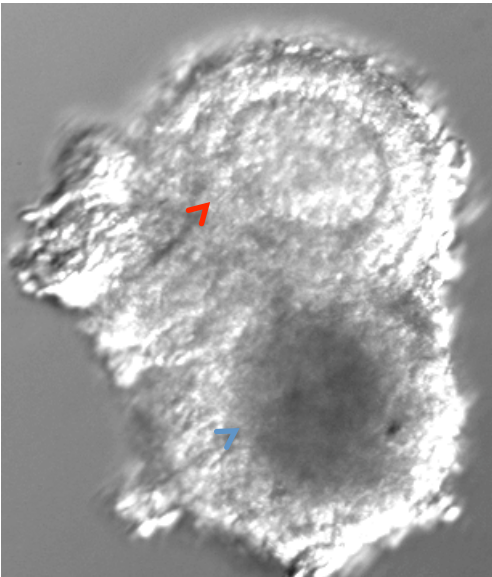
## Primary and Primordial follicles



Primordial follicle (black arrow) with less than one layer of granulosa cells. Primary follicle (red arrow) with one full layer of granulosa cells. All three follicles have impaired oocyte. You would not select these follicles for culture.

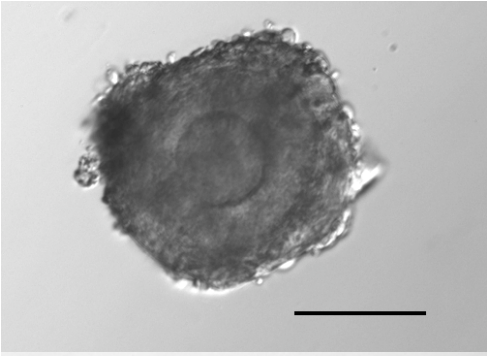


Two primary follicles (black arrow) and two early secondary follicles (red arrow). Although the primary follicles are healthy and round, they are too small to successfully grow. All four follicles have normal oocytes. The secondary follicles could be selected for culture and would be expected to grow.



One primary follicle (red arrow) and a dark follicle (blue arrow) surrounded by stromal cells. You would not select these follicles for culture because they are both primary and also because the dark follicle is unhealthy.

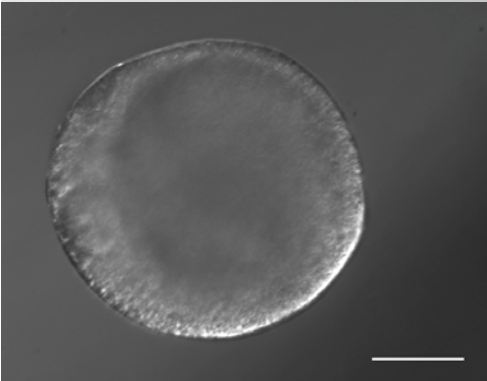
## Good Follicle Growth



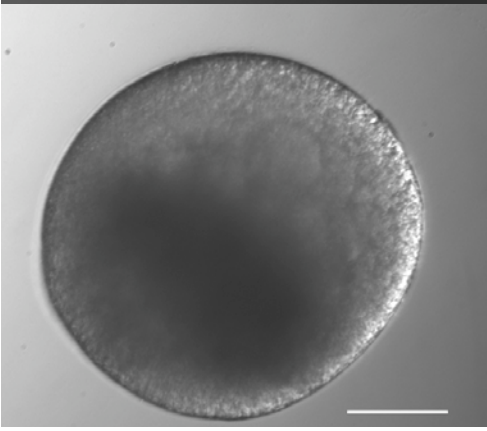
Day 0, mechanically isolated. 130um



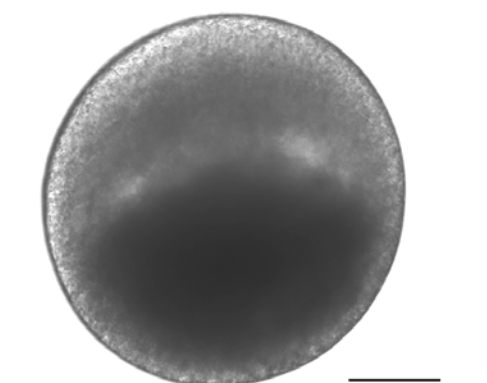
Day 2, follicle has reached 196um.  
Oocyte is still visible, round and healthy,  
layers of granulosa cells have increased.



Day 4, 325um. Oocyte still visible at lower  
left region of follicle, surrounded by antral cavity



Day 6, 365um. Oocyte visible  
at upper right-hand corner.



Day 8, 391um. Oocyte visible in upper central  
region of the follicle. All scale bars shown are  
100um. Day 0- Day 8 growth: 261um.