Neo-oogenesis: myth or reality?

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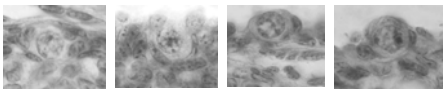
Ovarian reserve: new insights for clinical management

In 2004, a very provocative study was published in Nature

Germline stem cells and follicular renewal in the postnatal mammalian ovary
J. Johnson, J. Canning, T Kaneko, JK. Pru & J L. Tilly
Nature 2004, 428:145-150.

In this study, the authors claimed that in the adult mouse ovary, neo-oogenesis takes place and originates from germline stem cells present in the OSE



However, these cells, considered as germline stem cells by Johnson, are resting follicles leaving the ovary as a component of the resting pool depletion (Byskov and Rasmussen, 1973, Byskov 2005)

One year later … the same team published the following study

Oocyte generation in adult mammalian ovaries by putative germ cells in bone marrow and peripheral blood.
Johnson J, Bagley J, Skaznik-Wikiel M, Lee HJ, Adams GB, Niikura Y, Tschudy KS, Tilly JC, Cortes ML, Forkert R, Spitzer T, Iacomini J, Scadden DT, Tilly JL
Cell 2005, 122:303-315.

36 h after a doxorubicine treatment, which destroy germinal cells within 24h, spontaneous regeneration of the immature follicle pool was observed

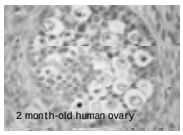
Consequently, Johnson et al. proposed again that neo-oogenesis takes place during adult life in the mouse ovary. However, egg cells no longer arise from the OSE but from bone marrow and other circulating cells.

These studies are very important, as they challenge the long-held view that mammals are born with a limited number of eggs that declines with age

However, in some primates, oogenesis continues after birth:

- In macaca fascicularis, OCT3/4, considered as exclusively expressed in premeiotic germ cells, can be detected 12 days after birth

- In humans, structures observed up to the age of 3 years, disappearing thereafter



- In prosimian monkeys, the adult ovary contains proliferating oogonia and meiotic oocytes in medullary nests *(Ioannou, 1967)*

Despite these observations, existence of a neo-oogenesis in the adult ovary can be disputed

In 2004 Johnson et al. said: " Incidence of follicular atresia is so high that ovaries should be almost devoid of healthy follicles in 42-day old mice. Consequently, neo-oogenesis explains why ovaries are not completely depleted at this age".

Which atretic follicles did Johnson et al. (2004) take into consideration?

" *Follicles at the* ***primordial, primary*** *and* ***preantral*** *(immature) stages of development were scored as atretic if the oocyte was degenerating, condensed or fragmented.*" Johnson et al. (2004)

Byskov et al. (2005) have injected 30-day-old mice (C57/Bl6 the same that in the johnson's study) with BrdU.

BrdU labels proliferating GC at the time of injection. If a given follicle becomes atretic, its GC remain labelled.

8 days after injection, the presence of an atretic oocyte corresponding to the Johnson's criteria in a labelled follicle means that this follicle was already present 8 days before

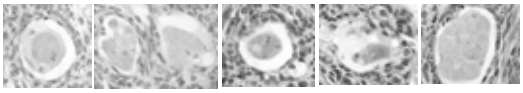
In the ovary of 30-day old mice, Johnson et al. counted around 2700 healthy follicles and 200 to 400 atretic follicles

Byskov et al., 2005					
	Primordial	Primary	Preantral	Antral	Late atresia
Healthy	1400-2700	150-235	215-275	45 -70	
Atretic	3 - 0	0	0	50 -110 *	185-370

* These atretic follicles contained healthy oocytes

Follicles at the late stage of atresia were labelled. Therefore, they were present in the ovary 8 days earlier, and were the only ones to present a degenerating, condensed or fragmented oocyte. Neither resting, nor early growing follicles showed involuting oocytes

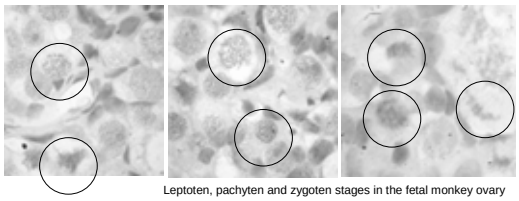
Conclusion: Johnson et al. (2004) did not count "*follicles at the* ***primordial, primary*** *and* ***preantral*** *(immature) stages of development*", but rather oocytes from follicles in very late atresia.



They did not apply the high clearance rate of 3h to 18h to a population of atretic resting or early growing follicles but, to a population of follicles in very late atresia that were already present several days (≥ 8) before.

Consequently, Johnson et al. have strongly overestimated the rate at which the pool of resting follicles depletes.
This suggests that adult female mice do not need neo-oogenesis for normal ovarian function.

Oogenesis is the process that transforms a proliferative oogonia into an oocyte through meiosis, it is followed by folliculogenesis.



Leptoten, pachyten and zygoten stages in the fetal monkey ovary

This process is slow, taking 4 days in the fetal mouse ovary (Peters et al., 1962), and probably much longer in humans

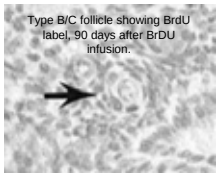
If Johnson's renewal follicle number was right, around 60 oocytes in transitory stages of meiosis would appear every day (Byskov)

Although we observed hundred thousands primordial follicles in at least 200 human ovaries, and similar numbers in the mouse, we never observed meiotic stages before the dictyate stage. We suggest that neo-oogenesis does not occur in the adult mouse/human ovary

Meredith et al. (2000) have shown that in rats, most resting follicles present at a given time are still present 150 days later

- 2 to 4-month-old rats were infused during 7 days with BrdU
- after infusion (day 0) rats were ovariectomized at day 0, 30, 90 and 150

Calculations revealed that almost 60 % of the follicles originally labelled as intermediary (type B/C) remained at this stage for the duration of the study

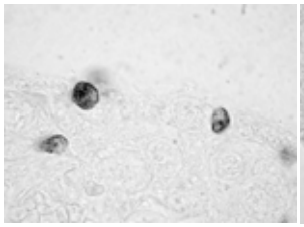


Type B/C follicle showing BrdU label, 90 days after BrDU infusion.

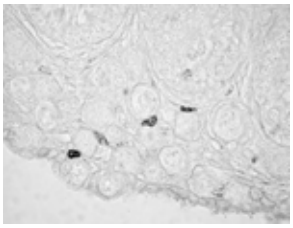
This study shows that most resting follicles remain at the same stage for up to 5 months. Their rate of atresia is therefore very low.

Consequently, the pool is not exhausted as quickly as suggested by Johnson et al., and replacement by new follicles generated by neo-oogenesis is not needed

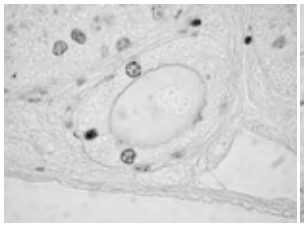
Absence of neo-oogenesis in the C57BL/6 mouse ovary between day 2 and day 30 of life (Byskov et al., unpublished data)



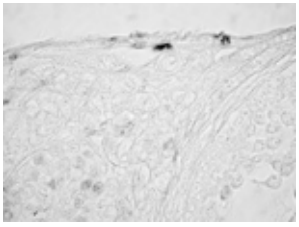
Day 2 (in the mouse, oogenesis is still progressing): 1h after the last BrdU injection. Meiotic oocytes (5%) are labelled



Day 10: 8d after BrdU injection. No labelled oocytes (400-500 sections/ 8 mice)



Day 30: 1h after the last BrdU injection. No labelled oocytes (400-500 sections/ 8 mice)



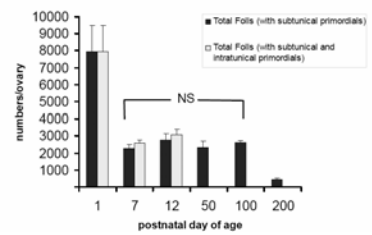
Day 38: 8d after the last BrdU injection. No labelled oocytes (400-500 sections/ 8 mice)

We cannot verify any neo-oogenesis using this test system

Finally, one morphological study supports the neo-oogenesis theory

Kerr JB, Duckett R, Myers M, Britt KL, Mladenovska T, Findlay JK. Quantification of healthy follicles in the neonatal and adult mouse ovary: evidence for maintenance of primordial follicle supply. Reproduction. 2006 *132*:95-109.

Healthy follicles in C57BL/6 mouse ovaries (the strain that Johnson et al. used) were quantified from day 1 to 200 using unbiased stereological methods.



In conclusion, despite the study by kerr et al. (2006) morphological studies do not argue in favor of neo-oogenesis in the adult ovary.

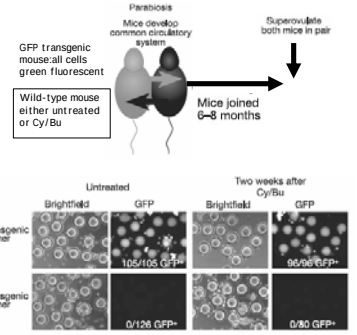
The two studies in Nature (2004) and Cell (2005) were followed by a strong debate (Albertini, 2004; Couzin, 2004, Gosden, 2004, Telfer, 2004, Greenfeld and Flaws, 2004; Vogel, 2005; Ainsworth, 2005; Powell, 2005; Martin, 2005; Byskov et al., 2005, Telfer et al., 2005; Hutt and Albertini, 2006),

In turn, some of these articles were followed by rough replies from Jonathan Tilly (Johnson et al., 2005).
« If you are still critical" said Tilly, *"get into the lab and prove us wrong"*"

From this time, some teams took Jonathan Tilly at his word: Eggan et al., 2006; Veitia et al., 2007; Liu et al., 2007. But more importantly, Tilly was the leader of a new very intriguing study (Lee et al., 2007)

The studies by Eggan et al. (2006) and Lee et al. (2007) constitute the most significant clarification in the neo-oogenesis controversy.

Eggan K, Jurga S, Gosden R, Min IM, Wagers AJ.
Ovulated oocytes in adult mice derive from non-circulating germ cells.
Nature 2006 *441* 1109-1114.



1st conclusion: There is no evidence that bone marrow cells or any other circulating cells contribute to the formation of mature ovulated oocytes

2nd conclusion: chemotherapy does not destroy all oocytes

- Cy/Bu treatment 2 weeks before superovulation slightly decreased the number of ovulated oocytes: 20 ± 6 vs 32 ± 13
- Cy/Bu treatment 2 months before superovulation strongly decreased the number of ovulated oocytes: 2 ± 1 vs 14 ± 4

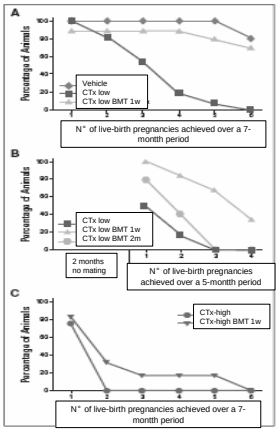
Non parabiosed Cy/Bu treated animals gave the same low numbers of ovulated oocytes, indicating that the reduced ovulation in Cy/Bu treated parabionts is not rescued by factors present in the circulation

In the second study

Lee H-J, Selesniemi K, Niikura Y, Niikura T, Klein R. Dombkowski DM, Tilly JL. Bone marrow transplantation generates immature oocytes and rescues long-term fertility in a preclinical mouse model of chemotherapy-induced premature ovarian failure. J Clin Oncol 2007 *22* 3198-3204.

Premature ovarian failure was induced by a mixture of cyclophosphamide and busulfan (CTx) in 6-week old mice.
A bone marrow transplantation (BMT) was made either 1 week or 2 months after chemotherapy.
Mice were mated either just after BMT or 2 months after BMT

Fertility was assessed by both the percentages of mice achieving live-birth pregnancies and the number of pregnancies



Results

BMT performed 1 week after CTx with immediate mating restored fertility

If mating was delayed 2 months, fertility rescuing was decreased

If both BMT and mating were delayed 2 months, fertility rescuing was poor.

When doses of CTx were high, fertility rescuing was poor

All offsprings were derived from the recipient germline

Donor-derived oocytes (1.4%) were only seen in immature follicles

In summary, these two studies show, among others, that ovulated oocytes do not arise from bone marrow or other circulating cells.

Regarding this issue, the following study is very interesting

Veitia RA, Gluckman E, Fellous M, Soulier J,
Recovery of female fertility after chemotherapy, irradiation, and bone marrow allograft: further evidence against massive oocyte generation by bone marrow-derived germline stem cells.
Stem cells 2007 *25* 1334-1335.

6 years after BMT, the patient had her first menstruation. She became pregnant at the age of 20 years and delivered a girl.

Analysis of polymorphic microsatellites revealed a genetic link between the girl and the mother, not the donor

In their first article, Johnson et al. (2004) claimed:" … this work has significant clinical implications related to therapeutic expansion of the follicle reserve as a mean to postpone normal or premature ovarian failure"

These clinical implications require that neo-oogenesis takes place in the human ovary

Liu Y, Wu C, Lyu Q, Yang D, Albertini DF, Keefe DL, Liu L.
Germline stem cells and neo-oogenesis in the adult human ovary.
Dev Biol 2007 *306* 112-120.

- Absence of early meiotic-specific or oogenesis-associated mRNA (*SPO11, PRDM9, SCP1, TERT and NOBOX*) in adult human ovaries.

and

- Absence of early meiocytes and proliferating germ cells (as previously mentioned by others)

Led to the conclusion that neo-oogenesis does not take place in the adult human ovary

Very interestingly, although 0CT3/4 is considered as exclusively expressed in premeiotic germ cells, Liu et al. observed it in primary oocytes. This observation questions the so-called cell-specificity of such or such protein.

General conclusions

The study by Lee et al (2007) remains very intriguing in that it shows fertility resuing in recipients treated by chemotherapy followed within one week by BMT and mating.
When BMT and/or mating were delayed, the fertility rescuing was strongly decreased. Importantly, high doses chemotherapy did not allow substantial fertility rescuing.

Finally, it could be possible that unidentified factors/cells present in BM, either stimulate resting follicles, not destroyed by the cytotoxic compounds, to enter growth phase, or initiate "putative" germinal stem cells, already present in the ovary, to undergo changes leading to a neo-oogenesis.

However, since 2004, Tilly has provided many contradictory assumptions

- Germline stem cells arise from the OSE (2004), then from BM or other circulating cells (2005), or from the ovary itself (2007)

- In the article in Cell, Johnson et al. (2005) said:" … ovaries of female mice receiving BMT after chemotherapy were histologically indistinguishable from those of untreated controls in that the ovaries of both groups contained a full spectrum of immature and mature oocyte-containing follicles (GFP oocytes) as well as CL indicative of normal ovulatory cycles."

- But in 2007 (Lee et al., 2007), he said "… donor BM-derived cells can generate immature GFP oocytes in the ovaries of recipient females following BMT, but … follicles housing these germ cells in the ovaries apparently do not progress past the preantral stage of maturational development.

Neverhteless, we must thank Tilly and his team who have performed a service to the field of ovarian biology by prompting re-examination of the ‘‘central dogma’’.

Finally, the primary question “Neo-oogenesis, myth or reality?” remains unanswered.
I feel that in normal conditions, neo-oogenesis does not take place in the adult ovary, however, experimental conditions such as BM or peripheral blood transplantations, might induce some unexplained events leading to fertility rescuing in experimentally-induced premature ovarian failure

Many thanks to Ann Grete Byskov for her helpful contribution