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Extra View

Mammalian neo-oogenesis and expression of meiosis-specific protein SCP3 in adult human and monkey ovaries

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The concept of neo-oogenesis and follicular renewal during adulthood in mammalian females, including humans, is a novel concept with major significance for ovarian physiology and mammalian reproductive biology. Previous observations from our laboratory demonstrated that mesenchymal cells in the tunica albuginea are bipotent progenitors for both granulosa and germ cells in adult human ovaries. In the present studies, we demonstrate that the antibodies against meiotic entry synaptonemal complex protein 3 (SCP3)—a marker for meiosis, showed reactivity with segments of tunica albuginea and ovarian surface epithelium, and in oocytes of some primordial follicles in functional human and monkey ovaries. These observations suggest that SCP3 is expressed in adult human and monkey ovaries. Preparation for meiotic activity may have already occurred at the level of tunica albuginea stem cells, and meiotic prophase activity may continue and terminate in oocytes of newly formed primordial follicles.

The origin of germ cells in adult females of higher vertebrates (birds and mammals) has been a matter of dispute for over one hundred years. There were, in principle, two views: the oocyte "storage" and "continued formation" theories.[1] The "storage" doctrine is based on the opinion that in higher vertebrates there is never any increase in the number of oocytes beyond those differentiating during fetal or perinatal ovarian development from embryonic (primordial) germ cells. The "continued formation" theory suggests that primordial germ cells degenerate and new oocytes originate during adulthood from cyclical proliferation of the ovarian surface epithelium (OSE) stem cells, at the same time as vast numbers of already-formed oocytes are eliminated through atresia (reviewed in ref. 2).

The currently prevailing "storage" dogma for higher vertebrates is based on an assumption that the process of oogenesis in the animal kingdom follows a uniform pattern with two main variants. One variant is that oogenesis continues either uninterruptedly or cyclically throughout reproductive life—e.g., most teleosts, all amphibians, most reptiles and conceivably a few mammals. The other variant is that the oogenesis occurs only in fetal gonads, and oogonia neither persist nor divide mitotically during sexual maturity—e.g., cyclostomes, elasmobranchs, a few teleosts, perhaps some reptiles, all birds, monotremes and almost all eutherian mammals.[3]

However, the "storage" doctrine maintaining that there is no formation of new oocytes in humans and other mammals after the fetal period, as compared to the continued formation of oocytes in adult frogs, seems contrary to an evolutionary point of view. In humans, there is a disadvantage of storing fetal oocytes for several decades before use, due to the high sensitivity of eggs to environmental hazards, contrary to the observed production of fresh oocytes during adulthood in lower vertebrates and invertebrates for the generation of healthy progeny. There is no other example where the evolution of a species is associated with such a retrogressive step. Therefore, the most problematic issue of the "storage" doctrine is how the oocyte storage from the evolutionary theory point of view is an advantage over that of frogs.

Regarding such possible oogenesis in adult human females, few studies have been performed seeking the presence of adult stem cells in ovaries. Our two reports hypothesize a mesenchymal-epithelial transition to explain how mesenchymal cells in the tunica albuginea serve as bipotent progenitors for both granulosa and germ cells.[4,5] We have shown that these mesenchymal cells differentiate into OSE cells through a mesenchymal-epithelial transition. Segments of the OSE directly associated with the ovarian cortex are overgrown with tunica albuginea and form solid epithelial cords, which fragment into small nests of primitive granulosa cells descending into the lower cortex. The putative germ cells (PGCs) originate via asymmetric division from OSE cells covering the tunica albuginea. The PGCs subsequently divide symmetrically and enter the adjacent cortical vessels. During vascular transport, these PGCs increase in size, and are picked up by nests of primitive granulosa cells associated with the vessels. During follicle formation, extensions of granulosa cells enter the oocyte cytoplasm, forming a single paranuclear Balbiani body that supplies the oocyte with additional

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mitochondria, known to be required for further growth and maturation. It has been observed that these studies "certainly deserve the merit of being the first reports to challenge the (current storage doctrine) consensus concerning human beings",[6] and that "time will tell whether the models offered by Bukovsky et al., are to be regarded as the start of 'unequivocal' evidence for human neo-oogenesis that has been lacking for more than 100 years".[7]

In midpregnancy human fetal ovaries, which have no tunica albuginea, the primitive granulosa cells and PGCs appear to originate from OSE precursors.[8,9] Pegs of proliferating OSE cells descend into the ovarian cortex forming primitive granulosa cells available for the formation of primordial follicles. The PGCs originate from asymmetrically dividing OSE cells, under the influence of immune system related cells (tissue macrophages and T cells), like in adult human[4,5] and rat ovaries.[10]

In a recent study, Liu and colleagues compared expression of meiotic entry synaptonemal complex protein-3 (SCP3) in fetal and functionally undefined adult human ovaries. The authors argued that SCP3 protein was not detectable in the tunica albuginea, OSE or in oocytes of primordial follicles in adult ovaries, and hence concluded that no meiotic oocytes are present in ovaries during adulthood.[11] In a subsequent commentary, Tilly and Johnson[12] indicated that the lack of evidence on neo-oogenesis in adult human females is not evidence of its absence, and on the contrary that some data of Liu et al.,[11] support the existence of neo-oogenesis in adult women. Here we report that using the same SCP3 antibody, immunoreactivity with segments of tunica albuginea and OSE, and in some oocytes of primordial follicles in functional adult human and monkey ovaries was detected.

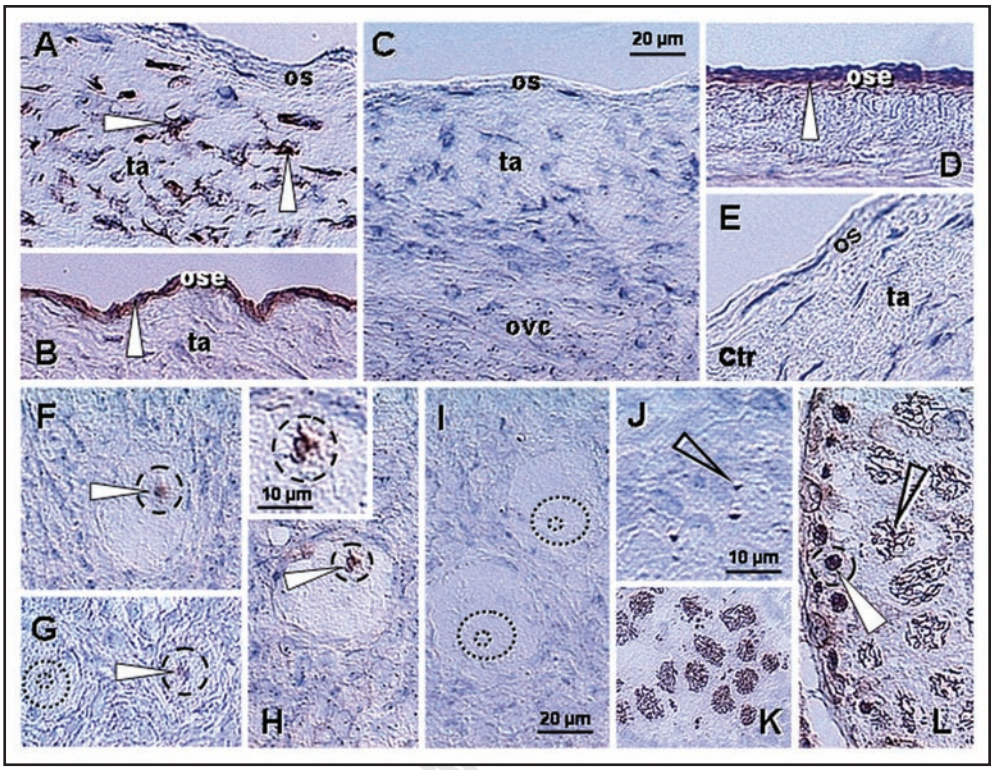

Figure 1. Immunohistochemical staining (brown color and arrowheads) for SCP3 expression (A–D and F–L). (A) Segments of tunica albuginea in ovaries with follicular renewal (early luteal phase) showed strong staining of mesenchymal (arrowheads) OSE precursors under ovarian surface (os). (B) Staining of OSE (ose and arrowhead) was apparent in other segments—note lack of staining of tunica albuginea under developed OSE. (C) Human ovaries in midluteal phase showed no staining of ovarian surface (top), tunica albuginea (ta) and ovarian cortex (ovc). (D) In postovulatory monkey ovaries, staining of OSE was also observed. (E) Control immunohistochemical procedure (Ctr) showed no staining. (F) Postovulatory human ovaries showed staining of nucleoli (arrowhead) in some primordial follicles. (G) In monkey ovaries, similar staining of nucleoli in some primordial follicles was observed (arrowhead). (H) Staining of paired chromosomes was observed in human ovaries (inset shows higher magnification). (I) Nucleolar or chromosomal staining was not apparent in all primordial follicles. (J) Testis of 3-day-old rat showed staining of some spermatogonial nucleoli (arrowhead). (K) In 20-day-old rat testes a chromosomal SCP3 expression was apparent in primary spermatocytes. (L) Adult rat testis (positive control) showed staining of condensed chromosomes in spermatogonia (solid arrowhead) and progression of meiotic division in primary spermatocytes (open arrowhead). Bar in (I) for (A–I; except inset in H) and for (K and L).

In functional ovaries with ongoing follicular renewal, the occurrence of unoccupied nests of primitive granulosa cells (often occupying a portion of the vascular lumen), oocyte/nest assembly, and degenerating superfluous oocytes in ovarian medullary vessels[5] were observed during the postovulatory (early luteal) phase of the menstrual cycle (see evidence of follicular renewal in Table 1, supplemental Figure S1A and B, and supplemental materials for Materials and Methods). In these ovaries a strong SCP3 expression was detected in some segments of tunica albuginea (Fig. 1A). Such tunica albuginea cells exhibited a mesenchymal pattern (arrowheads), which is characteristic for the OSE precursors.[5] The differentiated OSE (ose, B) also showed SCP3 immunostaining (arrowhead).

However, functional ovaries without evidence of ongoing follicular renewal (midfollicular, mid- and late luteal phases) lacked staining for SCP3 in tunica albuginea and at the ovarian surface (C). The SCP3 immunoexpression was also evident in differentiated OSE of postovulatory monkey ovaries (D). Control immunohistochemical procedure produced no staining (E).

Moreover, the SCP3 immunostaining was observed in the nucleoli of oocytes in some primordial follicles in human (arrowhead, F) and monkey ovaries (arrowhead, G). Recently, Tres reported that male germ cells exhibit nucleolar SCP3 expression during early stages of meiotic prophase.[13] In addition, an SCP3+ synapsis of two chromosomes was detected in human primordial follicle oocytes (arrowhead, H and insert), possibly representing XX chromosomal synapsis, since sex chromosomes start synapsis during early zygotene, before autosomes synapse.[13]

Observations from twelve human cases studied are summarized in Table 1. Rare SCP3+ oocytes (less then 10%) were detected in midfollicular phase ovaries (case #1). The most frequent expression

Table 1 **Menstrual cycle stage and SCP3 expression in the oocytes of primordial follicles (PF)**

Case #	Menstrual cycle stage[a]	Age	Evidence of follicular renewal[b]	PF oocytes with SCP3 expression[c]
1	MF	38	no	rare
2	EL	28	yes	+
3	EL	30	yes	+
4	EL	33	yes	+
5	EL	35	yes	+
6	EL	35	yes	+
7	EL	38	yes	+
8	EL	42	no	none
9	ML	27	no	rare
10	ML	34	no	none
11	LL	38	no	none
12	PCO	38	no	none

[a]MF, midfollicular; EL, early luteal; ML, mid luteal; LL, late luteal; PCO, polycystic ovaries; [b]Nests of primitive granulosa cells, oocyte/nest assembly, degenerating superfluous oocytes in some ovarian medullary vessels (reviewed in Ref. 5 for details); [c]Rare = less than 10%; + = 10 to 30%

(10 to 30% of primordial follicle oocytes) was found in postovulatory ovaries during the early luteal phase in younger (up to 38 years of age) women (cases #2–7). However, at the age of 42 years (case #8), postovulatory ovaries showed no SCP3 expression. Unstained oocytes (Fig. 1I) accompanied SCP3+ meiocytes. Virtually no staining of oocytes was observed in three younger women studied during the mid- and late luteal phases (cases #9–11; except for the rare occurrence in case #9) and in PCO ovaries (case #12). J–L in Figure 1 show SCP3 expression in developing rat testes (positive control). Note SCP3 immunostaining of spermatogonial nucleoli in the 3-day-old rat (arrowhead, J), which is characteristic for early stages of meiotic prophase[13] and resembles observations in primordial follicle oocytes of postovulatory adult human and monkey ovaries (see Fig. 1F–H). During further development, chromosomal staining of primary spermatocytes was apparent on day 20 (K), and condensed chromosomes in spermatogonia (solid arrowhead, L) and advanced progression of meiotic division in primary spermatocytes (open arrowhead) was observed in 2-month-old male gonads.

Using immunohistochemistry, Liu et al.,[11] concluded that they were unable to detect staining for SCP3 in tunica albuginea, OSE and in oocytes of primordial follicles in six cases of adult functionally undefined ovaries vs. positive fetal controls. It must be understood, however, that ongoing fetal oogenesis is a continuous process, while during adulthood it is confined to the postovulatory period in normal rodent and human ovaries.[1,5,14] The adult cases in the study of Liu and collaborators included women with ovarian biopsies for unknown reasons, and ovariectomy due to uterine myomas and focal endometrial cancer. The samples were not defined regarding ovarian ovulatory function, as no evidence was provided about the presence or absence of developing or regressing corpus luteum, or the phase of the menstrual cycle confirmed by endometrial biopsy. Furthermore, no higher magnification images were provided to study SCP3 expression in detail.

By utilizing the same commercial SCP3 antibody, we were able to detect immunoreactivity within segments of tunica albuginea, OSE and primordial follicle oocytes in sections from functional adult human and monkey ovaries. However, even in postovulatory ovaries 70 to 90% of oocytes were unstained. This suggests that SCP3 expression may be characteristic of new oocytes periodically complementing the existing pool which is continually depleted by transformation into growing follicles and by atresia.[15] Our observations indicate that preparation for meiotic activity may already occur at the level of tunica albuginea stem cells, and meiotic prophase activity may continue and terminate in new oocytes. However, the SCP3 staining was reserved for the postovulatory period and was not observed in polycystic anovulatory ovaries of younger women (age 38) and in functional aging ovaries (age 42). This suggests that oocyte and follicular renewal may occur in functional ovaries during the prime reproductive period [(PRP) till 38 ± 2 years of age—reviewed in ref. 2] only.

In addition to immunohistochemical studies, Liu and colleagues presented data derived from gene expression studies of early meiotic-specific or oogenesis associated mRNAs in ovaries of four human females of 28, 36, 41 and 53 years of age, using 26 week fetal ovaries as controls. Only two of these four women were in the age of the PRP, where the incidence of follicular renewal can be expected in about 33% of women with regular ovulatory cycles, during the postovulatory phase in particular, and in some ovarian segments only.[5] Hence conclusions of Liu and colleagues from gene expression studies[11] were based on two undefined cases (except age). Data from RT-PCR analysis of SCP3 in adult human ovaries contradict the authors' conclusions on the absence of meiosis in adult human ovaries. The fetal ovary showed strong SCP3 expression in RT-PCR, due to the widespread oogenesis in midtrimester fetal ovaries. Although at a lower level, the SCP3 gene expression was also apparent in all four adult ovaries. Such observations corroborate neo-oogenesis in adult human ovaries.[12] Of particular interest is the SCP3 gene expression in pre- (age 41) and perimenopausal (age 53) ovaries, which indicates that formation of new germ cells could continue after the end of PRP, although conditions required for the formation of new primordial follicles, e.g., formation of primitive granulosa cell nests,[5] may be terminated.

By Western blot analysis, Liu et al. found SCP3 protein expression in human fetal but not adult ovaries. However, the number of oocytes in adult human ovaries during PRP (6×10^4, reviewed in ref. 16) is 0.1% of the oocyte pool in midtrimester human fetuses (7×10^7, reviewed in ref. 17). Hence the 1000x less SCP3 protein in adult ovaries may need 1000x more ovarian protein load for SCP3 to be detected by Western blot.

The "storage" doctrine as elaborated by Sir Solly Zuckerman and collaborators (reviewed in ref. 3) is based on the following milestones (assumptions):

(1) Total number of oocytes declines with age by a simple regression.
(2) Oocytes persist in rat ovaries lacking OSE.
(3) Oogonia do not persist in adult ovaries.
(4) Oogenesis from somatic stem cells is missing.
(5) Mitotic division of oogonia is missing.

Regarding assumption (1), there is no significant decline between 18–38 years of age in humans.[16] In addition, Faddy[18] indicated that the pattern of primordial follicle number decline is not exponential, but more bi-exponential corresponding to a 'broken-stick' regression of logged total numbers of follicles against age. Such a model implies an abrupt change in the exponential rate of follicle loss at age 38 years, and is thus rather implausible biologically.[18] The model will

be biologically plausible when follicular renewal is considered to act before (slow decay rate during oocyte renewal) but not after 38 years of age (fast decay rate during oocyte storage).

Regarding the argument (2) that OSE is not essential for-neo-oogenesis since the oocytes persist in ovaries lacking OSE, we recently demonstrated that in rat ovaries lacking OSE, the oocytes originate by an alternate pathway, from medullary somatic stem cells; primordial follicles are formed in the juxtaposed ovarian cortex.[10]

Assumption (3) is in principle correct, since the oogonia should not persist in adult ovaries, due to the threat of accumulation of genetic anomalies with age. Yet, in adult human females, precursors of germ cells are tunica albuginea stem cells,[5] which have a mesenchymal character and are certainly more resistant to environmental threats and to the accumulation of genetic abnormalities with age.

Regarding point (4), step by step oogenesis and follicular renewal from somatic stem cells have been described in fetal and adult human and adult rat ovaries.[4,5,8,10]

Finally, regarding query (5), the mitotic division of newly formed germ cells and oogonia has been described in human and rat ovaries.[5,10]

Regarding both the storage and continued oocyte formation paradigms, there appears to now be a consensus that germ cells *per se* do not persist in adult mammalian ovaries from the fetal/perinatal period. From the view of groups attempting to re-establish the continued formation doctrine and search for the origin of new germ cells in adult humans and laboratory rodents,[4,5,10,14] there appears to be a consensus that during adulthood the germ cells originate from progenitor cells. Two possible mechanisms for the generation of new oocytes in postnatal mammals have been recently proposed by Joshua Johnson:[7]

(1) New oocytes are produced via germ stem cells that reside in an extragonadal location, the bone marrow, and are released into the peripheral blood. These progenitors migrate to the ovary, where they may engraft as new oocytes within new follicles.[14] The developmental potential of labeled oocytes after bone marrow transplantation remains unclear.[19]

(2) New oocytes are produced by a transformative mechanism. Ovarian bipotential progenitor cells produce both new oocytes and somatic (granulosa) cells within the ovary.[4,5,20]

Most recently, it has been reported that bone marrow transplantation improves attenuated fertility after low dose chemotherapy in mice, although all newborns were of recipient and not of bone marrow donor origin.[21] Tilly's group introduced in 2004 the idea of the origin of female germ cells in mice from persisting germline stem cells in the ovary.[22] A year later, this was replaced with the idea of the extra ovarian origin of mouse PGCs from bone marrow.[14] They also now found the idea on the origin of germ cells from bone marrow untenable, suggesting that bone marrow cells function primarily by reactivating host oogenesis impaired by chemotherapy.[21] However, they did not indicate which bone marrow cells are involved and how and where the new germ cells originate in the recipient. Our studies suggested that the bone marrow derived white blood cells (monocytes and T lymphocytes) accompany the origin of new germ cells from OSE stem cells in fetal and adult human and adult rat ovaries, or from medullary stem cells in adult rats lacking OSE (reviewed in ref. 2) Furthermore, activated resident vascular perictyes and bone marrow derived monocytes accompany initiation of follicular growth, selection and preovulatory maturation of autologous ooctyes.[2,4,9] We propose that the lack of activated pericytes and bone marrow derived monoctyes committed for the stimulation of follicular growth and maturation of the donor oocytes may be why the primordial follicles formed from circulating donor germ cells do not mature and ovulate, as recently reported.[14,19,21]

During certain periods of life the storage of oocytes may occur. Recently, we attempted to establish a harmony between the "storage" and "continued formation" theories by proposing the "prime reproductive period" theory[2,10,23] as follows: The "storage" theory pertains to two periods of the life in human females, that is between the termination of fetal oogenesis and puberty or premenarcheal period (about 10 to 12 years), and premenopausal period following the end of the PRP until menopause (also about 10 to 12 years). On the other hand, the "continued formation" theory accounts for the follicular renewal during the PRP (about 25 years, i.e., between menarche and 38 ± 2 years of age), and ensures an availability of fresh oocytes for the development of healthy progeny. Since the number of primordial follicles begins to diminish in aging rodents,[24] one may consider the relevance of the PRP theory in these species as well.

In conclusion, we are convinced that the neo-oogenesis and follicular renewal during the PRP exists throughout the animal kingdom, including higher vertebrates.

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Note

Supplementary materials can be found at:
www.landesbioscience.com/supplement/BukovskyCC7-5-Sup.pdf

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