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COMMENTARY

Unifying theory of adult resting follicle recruitment and fetal oocyte arrest

Sherman Silber *

Infertility Center of St Louis, St Luke's Hospital, 224 South Woods Mill Road, Saint Louis, MO 63017, USA
* *E-mail address:* silber@infertile.com.

Abstract One of the biggest mysteries of ovarian physiology is what controls the emergence of adult primordial follicles from the resting stage, and their steady depletion over the woman's lifetime. A related mystery is why do early oogonia begin meiosis in the fetus and then suddenly arrest for most of fetal and adult life. If fetal oocyte arrest did not occur after meiotic activation, there would be no oocytes left in the female baby by the time she is born. Similarly, without a steady controlled release in the adult ovary of resting follicles, the adult woman would run out of her eggs prematurely and have an early menopause. Could there be a similarity between what causes fetal oocyte arrest and what causes adult oocyte recruitment? The answer begins with the observation of a sudden massive recruitment of primordial follicles after human ovarian transplantation, and the embryologic discoveries about oocyte activation and the time of differentiation of cortex and medulla. The unifying theory is that ovarian cortical tissue pressure controls both fetal oocyte arrest and adult oocyte recruitment.

KEYWORDS: AMH, follicles, oocyte, ovarian, ovary, recruitment

Could there be a similarity between what causes fetal oocyte arrest and what causes adult oocyte recruitment? The sudden massive recruitment of primordial follicles and increased anti-Müllerian hormone (AMH) levels after human ovarian transplantation, with eventual loss of huge numbers of follicles but continued ovarian function, would support a theory that ovarian follicle recruitment with resumption of meiosis in adults and meiotic arrest in the early fetal ovary may be related events (Silber et al., 2015). For example, the early clinical event of massive resting follicle recruitment after frozen as well as fresh ovarian cortex grafting, with massive AMH elevation (following FSH decline) from about 140 to 280 days indicates a rapid 'escape' of massive numbers of adult primordial follicles from their 'resting phase' in the transplanted ovarian cortex, a phenomenon that is never seen in the normal ovarian state (**Figure 1**).

Interestingly, the latest data on the mechanism of fetal oocyte meiotic activation and arrest, we think, fits well with our observation in adults (Baltus et al., 2006; Byskov and Andersen, 2013; Byskov et al., 1997; Koubova et al., 2006, 2014; Rajah et al., 1992).

The occurrence of endocrine function and pregnancy after fresh and frozen ovary transplant is of great clinical value in itself; however, an interesting phenomenon also observed in these cases is that the FSH comes down to normal levels by 140 days in all cases, as would be predicted by Gougeon (1986). Although ovulation resumes in all cases, at the same time, the AMH level soars way above normal levels, representing massive oocyte recruitment. By about 280 days, the AMH of the recipient descends to well below baseline levels, and then remains steady at this level usually for many years. Therefore, follicle loss comes later, not from ischaemia but apparently from over-recruitment (Silber et al., 2015).

There must be a mechanism (non-hormonal) for controlling the steady modest release of 1000 resting follicles per month in the normal adult ovary in contrast to this massive

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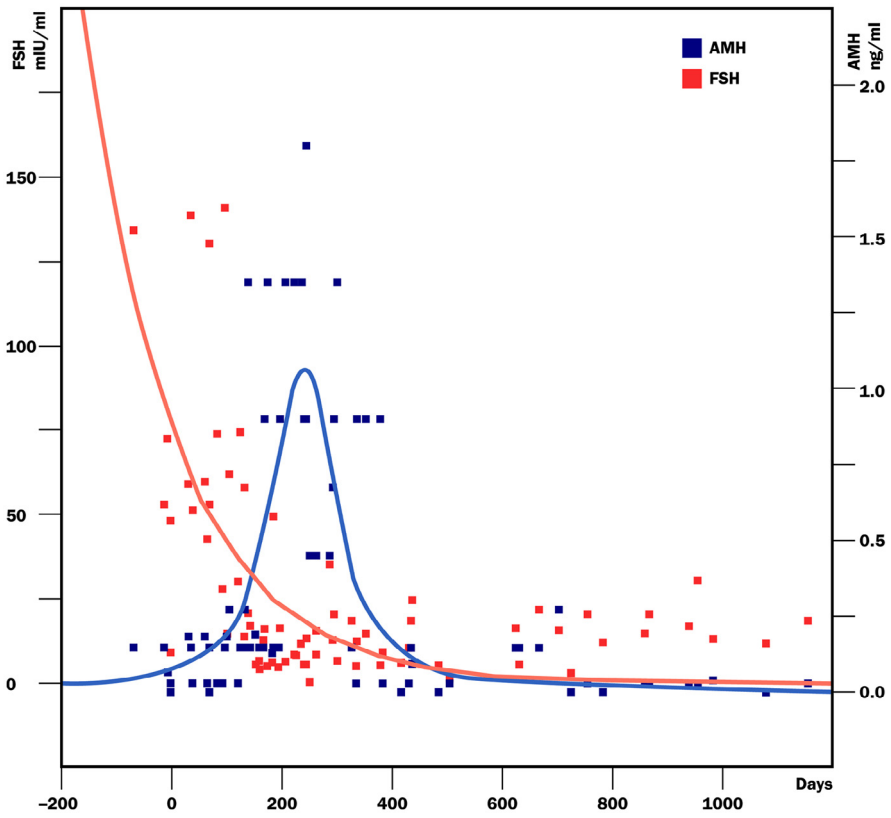

Figure 1 Levels of FSH and anti-Müllerian hormone (AMH) levels for women who underwent cryopreserved ovary transplantation. Women reported laboratory results to the centre on a monthly basis (on average). Blood was drawn on day three of the woman's cycle and follow-up continued throughout the functional life of the ovarian tissue. The best fit curves show a clear increase in AMH levels (mean = 0.43, SD = 0.52) before their return to normal as well as the decrease in FSH levels (mean = 34.49, SD = 40.35) (Silber et al., 2015).

over-recruitment of follicles after ovarian cortical transplant. Similarly, in the fetus, a massive transportaton of oocyte primordial germ cells from the epiblast to the genital ridge occurs and meiosis is initiated in these fetal oocytes caused by a flooding with retinoic acid from the adrenal gland and subsequent Stra 8 up-regulation from the adrenal gland. In fetal males, the Sertoli cells of the testis block the meiosis stimulating effect of retinoic acid, and so, in the male fetus, the germ cells do not enter meiosis (**Figure 2**). Meiosis in the fetal oocytes should continue to completion in all these oocytes, and you might think that the baby would be born without any eggs remaining. Similarly you might also think the adult would run out of eggs quickly and permanently. But the fetal oocytes remain arrested in meiosis, although, even in the fetus and in the baby and young girls, oocytes gradually reduce from 6 million in the fetus to 2 million at birth to about 400,000 in the young adult. What controls the rate of this adult oocyte loss, and is this rate of loss just emergence from the original fetal oocyte arrest?

The stroma of the male tunica albuginea (which is the same as the female ovarian cortex) is the toughest and most dense tissue in the body (**Figure 2**). It is quite possible that the tissue pressure inside the dense stroma in the female ovarian cortex arrests the continuation of early meiosis of the oocyte so that females do not lose all their eggs at once, and also provides a steady release mechanism of eggs for the female adult. No hormonal control has yet been detected. The primordial follicles in the adult simply escape arrest and develop toward the softer interior and into the very soft medulla. It is all possibly regulated just by tissue pressure. All follicular development in the adult begins at the softer interior aspect of the cortex and continues toward the very soft medulla where it completes its maturation and, finally after 4 months, becomes sensitive to LH and FSH. Anatomic studies of the early embryonic ovary show that oocytes in the medulla die off and those in the cortex are preserved. In fact, most likely what we call resting follicle recruitment is just 'escape from fetal arrest', and all may simply be governed by pressure. As primordial germ cells become oogonia under the influence of the ovary, they are stimulated to initiate meiosis by retinoic acid, which upregulates the gene Stra 8, the meiosis-inducing gene. But if they continued in meiosis, the eggs would all be depleted by the time of birth. That is why they must be arrested in early meiosis and their escape from this arrest must be controlled in some fashion. When they are recruited in adult life to develop into secondary follicles and eventually ovulatory follicles, they are, in fact, just escaping from their 'arrested' fetal state.

Our adult ovarian transplant results support a unified theory in which meiosis of the oocyte is initiated by retinoic acid in the fetus when oogonia arrive at the genital ridge but then arrested and maintained in arrest by the pressure of the ovarian cortex (Byskov and Andersen, 2013; Byskov et al., 1997). The tunica albuginea of the testes is the most dense fibrous

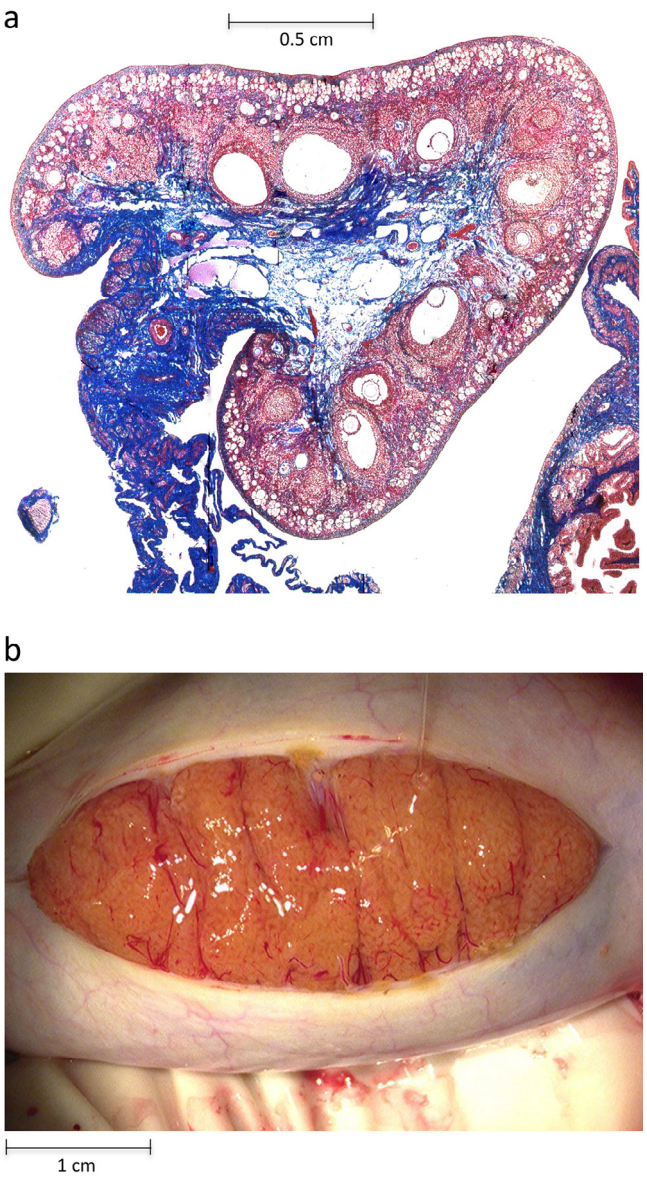

Figure 2 (a) Adult ovary: primordial follicles leave the dense cortical stroma and develop into maturing follicles toward the softer centre of the ovary; (b) adult testicle: the tunica albugina of the testis is the very dense tissue that corresponds to the dense stroma of the ovarian cortex.

tissue in the body, and the ovarian cortex is its female homologue (Morris Textbook of Anatomy; Morris, 1966). In all cases of ovarian development in mammals, the first meiotic germ cells are situated towards the medulla, and the oogonia in the cortical periphery are the last to enter meiosis (Byskov and Andersen, 2013); only the oocytes that arrive to the cortex are arrested and thus salvaged. In the ovarian cortex, a gradient of stromal density exists, with the most tissue pressure and density being near the surface, gradually decreasing towards the centre, with the least dense tissue in the ovarian medulla (**Figure 2**) (Shikanov et al., 2011; Silber et al., 2015; Woodruff and Shea, 2011). With the use of in-vitro follicle culture, it has already been postulated that pressure may hugely affect resting follicle development (Shikanov et al., 2011; Woodruff and Shea, 2011). Also, it is well known that the 'resting' (or arrested) adult primordial follicles are recruited first from the least dense internal stroma of the cortex and develop toward the medulla, which is the least dense tissue of the ovary. The stromal density reduces further inward from the surface of the ovarian cortex. Then, once the follicles become relatively huge Graafian follicles, and ready to ovulate, they burst out through the surface from the medulla to ovulate.

Evidence for this is strong in the fetal mouse, where the time period around meiotic initiation is the key period for setting up growing follicles in the medulla and non-growing follicles in the cortex (Byskov and Andersen, 2013; Byskov et al., 1997; Koubova et al., 2006; Rajah et al., 1992). This observation in fetal mouse ovaries corresponds well to our clinical observation of very high AMH levels and massive follicle recruitment in early ovary transplants. If they are not trapped in the dense cortex, many follicles exit meiotic arrest and begin to grow. In fact, this could explain the remarkable result reported by Kawamura et al. (2013) of rejuvenating menopausal ovarian cortex with autotransplantation. When you remove ovarian cortex of a young patient with premature ovarian failure, a few follicles are usually trapped within that tough cortex. If you cut it up, release the pressure and transfer it back, suddenly you get recruitment. Oocyte meiotic arrest in the fetus at 8 weeks could be similar to release from primordial oocyte recruitment in the adult, all of which is mediated by tunica albuginea pressure, so women are not born without eggs, and the adults do not lose all their eggs before child-bearing age. Therefore, it could be that stromal density governs resting follicle recruitment, and which allows the fetal ovarian cortex to arrest the oogonia in early meiosis, and which accounts for the massive AMH release after ovarian cortical transplantation (Shikanov et al., 2011; Silber et al., 2015; Woodruff and Shea, 2011).

Our results support a unifying theory that tissue pressure may govern adult resting follicle recruitment, and may also allow the fetal ovarian cortex to arrest oogonia in early meiosis.

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References

Baltus, A.E., Menke, D.B., Hu, Y.-C., Goodheart, M.L., Carpener, A.E., de Rooij, D.G., Page, D.C., 2006. In germ cells of mouse embryonic oaries, the decision to enter meiosis precedes premeiotic DNA replication. Nat. Genet. 38, 1430–1434.

Byskov, A., Andersen, C.Y., 2013. Ontogeny of the mammalian ovary. In: Trounson, A., Gosden, R., Eichenlaub-Ritter, U. (Eds.), Biology and Pathology of the Oocyte. Cambridge University Press, Cambridge, UK, pp. 12–23.

Byskov, A.G., Guoliang, X., Andersen, C., 1997. The cortex-medlla oocyte growth pattern is organized during fetal life: an in-vitrostudy of the house ovary. Mol. Hum. Reprod. 3, 795–800.

Gougeon, A., 1986. Dynamics of follicular growth in the human: a model from preliminary results. Hum. Reprod. 1, 81–87.

Kawamura, K., Cheng, Y., Suzuki, N., Deguchi, M., Sato, Y., Takae, S., Ho, C.H., Kawawamura, N., Tamura, M., Hashimoto, S., Sugishita, Y., Morimoto, Y., Hosoi, Y., Yoshioka, N., Ishizuka, B., Hsueh, A.J., 2013. Hippo signaling disruption and Akt stimulation of ovarian follicles for infertility treatment. Proc. Natl. Acad. Sci. U.S.A. 110, 17474–17479.

Koubova, J., Menke, D.B., Zhou, Q., Capel, B., Griswold, M.D., Page, D.C., 2006. Retinoic acid regulates sex-specifictiming of meiotic initiation in mice. Proc. Natl. Acad. Sci. U.S.A. 103, 2474–2479.

Koubova, J., Hu, Y.-C., Shattacharyya, T., Soh, Y.Q., Gill, M.E., Godheart, M.L., Hogarth, C.A., Grlsworld, M.D., Page, D.C., 2014. Retinoic acid activates two pathways required for meiosis in mice. PLoS Genet. 10, 1–9.

Morris, H., Anson, B.J., 1966. Morris' Human Anatomy: A Complete Systematic Treatise. Blakiston Division. McGraw-Hill, New York.

Rajah, R., Glaser, E.M., Hirshfield, A.N., 1992. The changing architecture of the neonatal rat ovary during histogenesis. Dev. Dyn. 194, 177–192.

Shikanov, A., Smith, R.M., Xu, M., Woodruff, T.K., Shea, L.D., 2011. Hydrogel network design using multifunctional macromers to coordinate tissue maturation in ovarian follicle culture. Biomaterials 32, 2524–2531.

Silber, S.J., Pineda, J., Lenahan, K., DeRosa, M., Melnick, J., 2015. Fresh and frozen ovary transplantation and resting follicle recruitment. Reprod. Biomed. Online 30, 643–650.

Woodruff, T.K., Shea, L.D., 2011. A new hypothesis regarding ovarian follicle development: ovarian rigidity as a regulator of selection and health. J. Assist. Reprod. Genet. 28, 3–6.

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