ELSEVIER

Molecular and Cellular Endocrinology 163 (2000) 53–60

MCE
Molecular and Cellular Endocrinology

www.elsevier.com/locate/mce

The primordial to primary follicle transition

J.E. Fortune *, R.A. Cushman, C.M. Wahl, S. Kito [1]

Department of Biomedical Sciences and Section of Physiology, College of Veterinary Medicine, T6-012B Veterinary Research Tower, Cornell University, Ithaca, NY 14853, USA

Received 16 July 1999; received in revised form 3 August 1999; accepted 9 November 1999

Abstract

The mechanisms that regulate the gradual exit of ovarian follicles from the non-growing, primordial pool are very poorly understood. A better understanding of the signals that initiate follicular growth in mammals, and of the conditions necessary for sustained growth of early preantral follicles in vitro, could have practical implications for contraception, alleviation of infertility, and regulation of the rate of follicle depletion (menopause). Our laboratory has developed two experimental systems that can be used to study factors involved in the activation of primordial follicles. In the first experimental system, small pieces of ovarian cortex, containing mostly primordial follicles, are isolated from fetal ovaries of cattle or baboons and cultured in serum-free medium. Under these conditions most primordial follicles become activated between 12 and 24 h of culture; their granulosa cells change shape, from flattened to cuboidal, and begin to express proliferating cell nuclear antigen (PCNA). During 7 days in culture, the newly-formed primary follicles and their oocytes increase significantly in diameter. This wholesale 'spontaneous' activation in serum-free medium is quite different from the much more gradual exit of primordial follicles from the resting pool that occurs in vivo and suggests that primordial follicles in vivo may be subject to a tonic inhibition of growth initiation or, alternatively, that some aspect(s) of the environment in vitro stimulates growth initiation. Recently we developed a second experimental system for studying activation of primordial follicles. Pieces of ovarian cortex from bovine or baboon fetuses were grafted beneath the developing chorioallantoic membrane (CAM) of 6-day-old chick embryos, a site known to support xenografted tissues. The cortical pieces were rapidly vascularized and histological analysis of pieces recovered after 2, 4, 7, or 10 days 'in ovo' revealed no increase in the number of primary follicles and maintenance of original numbers of primordial follicles. Therefore, grafting ovarian cortical pieces beneath the chick CAM provides an experimental system in which follicles remain at the primordial stage in a readily accessible environment and which, thus, may be used to study potential regulators of the initiation of follicle growth. The results suggest that vascularization of isolated pieces of ovarian cortex provides conditions that maintain follicular quiescence, whereas culture in vitro allows unrestrained activation of primordial follicles. Future studies with and comparisons of the in vitro and in ovo models may provide new insight into the mechanisms that regulate the primordial to primary follicle transition.

Keywords: Ovarian follicle; Primordial follicle; Primary follicle; Follicular development; Ovary; Cattle; Baboon

1. Introduction

When ovarian follicles are formed they enter the resting, primordial stage which persists for a period of time that varies from follicle to follicle. The mechanisms responsible for the initiation of follicular growth, also known as primordial follicle activation, and the mechanisms that permit variable timing of growth initiation are completely unknown. The pool of non-growing, primordial follicles is a resource that could be utilized or manipulated to alleviate infertility, produce contraception, or delay menopause — if only we understood the signals that initiate follicular activation and the factors necessary to sustain the early growth stages.

The formation of primordial follicles, which have an oocyte in first prophase of meiosis and a single layer of flattened granulosa cells, occurs synchronously within a few days after birth in rats and mice (see Hirshfield, 1991 for review). Activation of a few primordial folli-

* Corresponding author. Tel.: +1-607-2533466; fax: +1-607-2533476.

E-mail address: jf11@cornell.edu (J.E. Fortune).

[1] Current address: NIRS, Division of Education and Scientific Services, 4-9-1 Anagawa, Inage-ku, Chiba 263-8555, Japan.

0303-7207/00/$ - see front matter
PII: S0303-7207(99)00240-3

cles begins almost immediately thereafter and yields the first group of antral follicles around day 15 postpartum (Hirshfield, 1991). Thus, the timing of follicle formation and the initiation of primordial follicle activation in rodents, coupled with an ovarian size that allows organ culture of whole ovaries, makes them very useful models. In contrast, follicle formation in ruminants and primates begins during fetal development and occurs over a long period of time, so that some follicles are activated and leave the resting pool before other follicles have been formed (Henricson and Rajakoski, 1959; van Wagenen and Simpson, 1965; Russe, 1983). These developmental processes are thus protracted and asynchronous in these larger species, making them more difficult to study. However, domestic animals and humans are the species of interest in terms of the practical goal of better regulation of female reproduction and it is, therefore, worthwhile to attempt to overcome the experimental problems that make it more difficult to study them.

In this manuscript, what is known about regulation of the primordial to primary follicle transition from experiments with rodents, cattle, and primates will be reviewed and some unpublished data from our laboratory will be presented. The two experimental models that our laboratory has developed for studying follicle activation and early follicular growth in cattle and baboons will be described and some of the results generated thus far with these models will be presented.

2. In-vitro models for studying the primordial to primary follicle transition

The activation of primordial follicles in vitro has been achieved for rodents, cattle, and primates. Blandau et al. (1965) cultured fetal mouse ovaries in serum-containing medium and reported that some oocytes grew in culture, specifically those that were surrounded by one or more layers of somatic cells. Eppig and O'Brien (1996) achieved the most dramatic success thus far with in vitro follicular activation. When they cultured newborn mouse ovaries, containing only newly formed primordial follicles, in medium with 10% fetal bovine serum (FBS) for 8 days, an apparently normal number of primordial follicles initiated growth and developed to the secondary follicle stage by day 8 (similar to development after 8 days in vivo). The secondary follicles were isolated for further growth in vitro and yielded fertilizable oocytes, embryos, and one live mouse. Thus, murine primordial follicles can initiate growth in vitro, at least in the presence of 10% FBS.

Our laboratory has developed a serum-free culture system that supports the activation of primordial follicles from cattle and baboons (Wandji et al., 1996, 1997). Since the larger size of ovaries of domestic species and primates precludes the whole-ovary culture system used successfully with rodents, we cultured small pieces of ovarian cortex (about $0.5 \times 0.5 \times 0.2$ mm), dissected from the ovaries of fetal calves or fetal baboons obtained during the last trimester of pregnancy. Primordial follicles reside in the outer, cortical region of mammalian ovaries and cortical pieces are, thus, enriched for primordial follicles relative to whole ovaries. Fetal ovaries are softer and easier to dissect than adult ovaries of ruminants or primates, which have a dense, tough stroma. The cortical pieces were cultured in Waymouth MB 752/1 medium supplemented with antibiotics, sodium pyruvate, and ITS + (Insulin–transferrin–selenium, BSA and linoleic acid) on transwell membrane inserts (see Wandji et al., 1996, 1997 for further details). Numbers of primordial, primary, and secondary follicles in freshly isolated cortical pieces and at various times during culture were determined by histological morphometry (Wandji et al., 1996, 1997).

As shown in Fig. 1, cortical pieces contained mostly primordial follicles and smaller numbers of primary follicles at the initiation of culture. However, by day 2 of culture the number of primordial follicles was reduced dramatically, concomitant with an increase in the number of primary follicles. Results for cattle and baboons were remarkably similar (Fig. 1) and suggest that primordial follicles in cortical pieces from both species had initiated growth in vitro in the absence of serum. This conclusion is supported especially well by the results of the studies with baboons, in which follicles were classified into additional categories (Fig. 1B and Wandji et al., 1997). This revealed a transient rise in early primary follicles, which have a partial layer of cuboidal granulosa cells (Fig. 1B), thus strengthening the conclusion that primordial follicles become activated early in the culture period and develop into growing primary follicles. The baboon cortical cultures were somewhat more successful than bovine cortical cultures in that the numbers of follicles classified as early secondary (partial second layer of granulosa cells) and secondary (full second layer) increased during culture (Fig. 1B). This was not entirely due to the longer culture period used for baboon cortical pieces since some secondary follicles were observed on days 4 and 7 of culture. Although some primordial and primary follicles in various stages of atresia were observed, the percentage of atretic follicles was not affected by culture (Wandji et al., 1996, 1997).

The massive exit of primordial follicles from the resting pool illustrated in Fig. 1 was unexpected and stands in contrast to the more normal number of follicles that initiated growth when newborn mouse ovaries were cultured (Eppig and O'Brien, 1996). The fetal source of the cortex used in our experiments with

bovine and baboon ovaries does not seem to be responsible for this difference because Braw-Tal and Yossefi (1997) reported a similar wholesale activation of primordial follicles when they cultured cortical pieces from adult cow ovaries for 2 days and the results of Hovatta et al. (1997) suggest that primordial follicles may activate in cultured slices of human ovarian tissue. Potential reasons for this difference between the results derived from cultures of ovarian cortex from cattle and primates and those from cultures of whole rodent ovaries will be discussed in a later section.

3. Chick chorio-allantoic membrane (CAM) grafts as a model for studying the primordial to primary follicle transition

Recently our laboratory has developed a second experimental model for studying the activation of primordial follicles; this model consists of grafting cortical pieces beneath the chorioallantoic membrane (CAM) of developing chick embryos. CAM grafts have been used for decades by embryologists to study the development of various organs and structures (reviewed by Rudnick, 1944; Rawles, 1952). Pieces of tissue placed on the CAM readily become vascularized and the lack of an immune system at this stage of chick development prevents graft rejection. First we tried the classical technique by grafting bovine ovarian cortical pieces, similar in size to those we typically culture, to the outer surface of the CAM of day 6 chick embryos. Fertilized chick eggs were incubated in an egg incubator under standard conditions (38°C, humidified). On day 4 after fertilization a small window was cut in the shell and masking tape placed over the window; on day 6, a freshly isolated bovine cortical piece ($0.5 \times 0.5 \times 0.2$ mm) was placed on the CAM. The grafts survived, but did not look healthy and were prone to desiccation. Next, we tried modifying the technique by placing the cortical piece beneath the edge of the developing CAM, between the CAM and the yolk sac membrane. The grafts were retrieved after 2, 4, 7, or 10 days of incubation and fixed for light microscopy. The CAM-grafted cortical pieces were analyzed by histological morphometry as described previously (Wandji et al., 1996) and compared with the results for cortical pieces from the same bovine fetuses that were cultured in vitro, as described in the preceding section.

As expected, in cortical pieces cultured in serum-free medium, the number primordial follicles decreased about 7-fold, concomitant with about a 6-fold increase in primary follicles, indicating that most primordial follicles had been activated in vitro (Fig. 2). In contrast, when cortical pieces from the same fetuses were transplanted beneath the CAM (placed 'in ovo'), numbers of primordial and primary follicles did not change significantly from numbers present at time 0 (Fig. 2). Therefore, the spontaneous and massive activation of primordial follicles that occurred in vitro was completely inhibited in ovo. Similar results were observed when cortical pieces from fetal baboon ovaries were grafted beneath the CAM (data not shown). Thus, we now have two different experimental situations, one (in vitro) in which most primordial follicles activate within a short time in culture and another (in ovo) in which spontaneous activation of follicular growth appears to be inhibited.

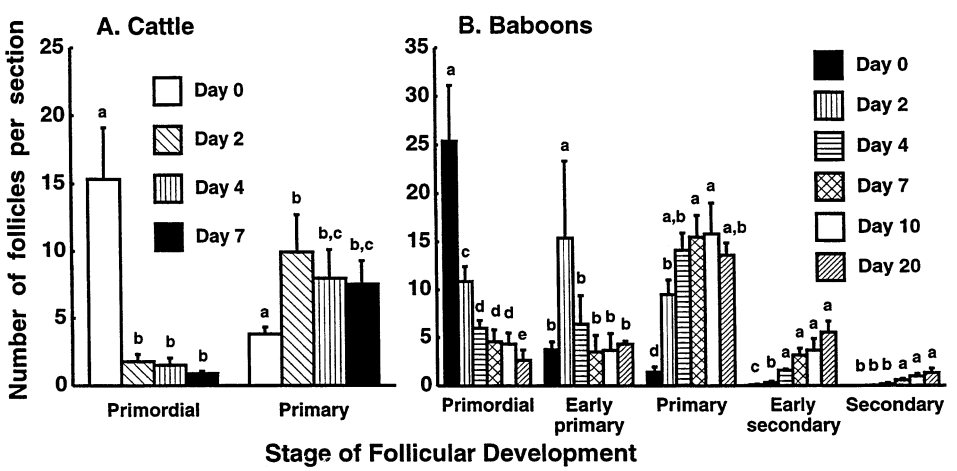

Fig. 1. Numbers of healthy follicles (mean per histological section ± SEM) in (A) fetal bovine ovarian cortex after 0, 2, 4, or 7 days in serum-free culture ($n = 4$ fetuses, with 59–71 sections examined per fetus; within each follicular stage, bars with no common superscripts are different: $a > b$, $P < 0.01$; $a > c$, $P < 0.05$). (B) Fetal baboon ovarian cortex after 0, 2, 4, 7, 10, or 20 days in serum-free culture ($n = 3$ fetuses, with 36–54 sections examined per day of culture; within each follicular stage, bars with no common superscript are different: $a > b$, $b > c$, $c > d$, $P < 0.05$; $a > c$, $a > d$, $a > e$, $b > d$, $c > e$; $P < 0.01$). (Reprinted from Fortune et al., 1998 with permission.)

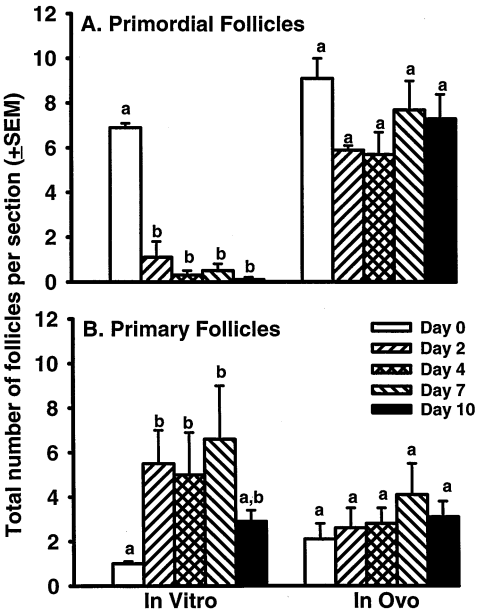

Fig. 2. Numbers of primordial and primary follicles in pieces of bovine ovarian cortex maintained in vitro in organ culture or as grafts beneath the chorioallantoic membrane of chick embryos (in ovo) for 0, 2, 4, 7, or 10 days. Pieces of fetal bovine ovarian cortex were isolated and cultured and numbers of primordial and primary follicles determined as described previously (Wandji et al., 1996). Twelve to 18 sections examined per fetus per time point ($n = 4$ fetuses). Data were log-transformed to remove heterogeneity of variance and then subjected to analysis of variance, followed by Duncan's multiple range test. Within each follicular stage, bars with no common superscripts are different: *a*, *b* $P < 0.05$).

4. What mechanisms regulate the primordial to primary follicle transition?

This question can be divided into two parts. First, what mechanisms stimulate or permit a follicle to enter the growth phase? Second, how are the mechanisms that stimulate or permit follicle activation regulated temporally, i.e. how is a gradual exit of follicles from the resting pool over the course of months or years achieved? The answers to these questions are unknown, but some hypotheses can be formulated based on what we do know. One idea is that follicle growth initiation may be regulated primarily by an inhibitor emanating from the more central medullary region of the ovary. If this were the case, it could explain why most primordial follicles activate when cortical pieces from cattle or baboons are placed in culture, since in that situation they are, presumably, released from the influence of a medullary inhibitor. In contrast, cultured whole mouse ovaries contain all ovarian components and would be able to continue to regulate the number of follicles leaving the resting pool. However, we have been unable to obtain direct support for the hypothesis of a medullary inhibitor. When cortical pieces were cultured with or without a piece of attached medulla, there was no difference in the rate of activation (Kito and Fortune, unpublished observations), but it could be argued that the concentrations of the putative medullary inhibitor might not be high enough in that situation. Another piece of evidence against the inhibitor hypothesis is the fact that when cortical pieces from bovine or baboon ovaries are grafted in ovo, spontaneous follicle activation does not occur. This argues against a medullary inhibitor as the regulator of follicle activation, unless the inhibitor or a similar molecule is present in the embryonic chick circulation. The failure of primordial follicles to activate in ovo does, however, provide evidence that simply separating the cortical region from the rest of the ovary is not sufficient to trigger activation.

Another hypothesis that we have advanced (Wandji et al., 1996, 1997) is that isolated ovarian cortical pieces may exhibit wholesale activation because their environment in vitro is actually richer (in nutrients, oxygen, etc.) than their environment was in vivo, since the cortical region of the ovary is poorly vascularized (Guraya, 1985; van Wezel and Rodgers, 1996). In ovo, the cortical pieces become vascularized very rapidly (at least by 2 days after grafting). They also become surrounded by membranes that form a bursa-like structure. Thus, the microenvironment is provided by the chick embryo vascular system and the 'bursal' fluid.

Hence, it is still unclear if activation of primordial follicles is regulated by an inhibitor, a stimulator, or a balance between inhibitory and stimulatory factors. Although a number of factors have been linked with early follicular growth (see section below), very few have been suggested as regulators of follicular activation. The only specific factor that has thus far been linked to the activation of primordial follicles is kit ligand (also called stem cell factor or steel factor). Kit ligand is produced by granulosa cells, whereas primordial germ cells, oocytes, and theca cells express the receptor for kit ligand, c-kit (Manova et al., 1993; Motro and Bernstein, 1993). In a recent report, Parrott and Skinner (1999) presented results that support a role for kit ligand in follicle activation in rat ovaries in vitro. Yoshida et al. (1997) injected mice with a function-blocking antibody to c-kit at various times during the first two weeks of life and concluded kit ligand is needed for the activation of primordial follicles, but not for their formation. An essential role for GDF-9 in follicular growth after the primary stage is indicated by the analyses of GDF-9 null mutants (Dong et al., 1996), but Bodensteiner et al. (1999) recently reported very low expression of mRNA for GDF-9 in primordial follicles of sheep and cattle and have suggested a role for this growth factor in the initiation of follicle growth in ruminants.

In vitro, primordial follicles in bovine and baboon cortical pieces activate within 2 days of culture. To

determine the timing and synchronicity of the massive and spontaneous activation that occurs when cortical pieces are cultured, we collected and fixed cortical pieces at 0, 12, 24, and 36 h of culture and analyzed follicular populations by histological morphometry. As shown in Fig. 3, the exodus of follicles from the resting pool occurs synchronously and abruptly between 12 and 24 h after they are placed in culture. The temporal preciseness of this transition from primordial to primary follicle in vitro suggests that a specific mechanism(s) affects simultaneously all follicles that are capable of activating in vitro. In future experiments, determination of biochemical and molecular changes that occur between 12 and 24 h of culture should provide insight into the mechanisms of the primordial-primary follicle transition.

5. Growth of follicles activated in vitro

Once follicles have undergone the primordial to primary transition in vitro, what is their potential for further growth in vitro? Although a great deal of research has been focused on methods for growing large preantral follicles in vitro, little is known about the conditions necessary to sustain and stimulate the smallest growing preantral follicles. This question is of considerable practical interest if the pool of resting primordial follicles is to be used, eventually, as a resource for alleviating infertility in women and propagating valuable domestic animals and members of endangered species. When we cultured ovarian cortical pieces from cattle and baboons, follicular activation was very rapid (Figs. 1 and 3), but the diameters of follicles and oocytes then increased in a more gradual and linear fashion (Fig. 4; Wandji et al., 1996, 1997). In addition to the increases in diameters evident in Fig. 4, immunohistochemical analysis for a marker of cell proliferation, proliferating cell nuclear antigen (PCNA), showed that few follicles exhibited any positive staining for PCNA at time 0, whereas by day 2 the newly activated follicles in both bovine and baboon cortical pieces stained strongly for PCNA (Wandji et al., 1996, 1997). Therefore, activation of primordial follicles is followed by growth of both the oocyte and follicle cells, as would be expected.

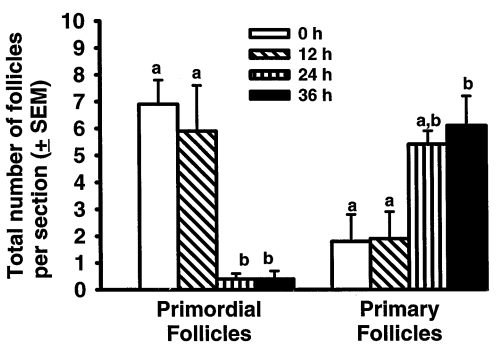

Fig. 3. Time course of activation of bovine primordial follicles during the first 36 h of culture. Pieces of fetal bovine ovarian cortex were isolated and cultured and numbers of primordial and primary follicles determined as described previously (Wandji et al., 1996). Twelve to 44 sections examined per fetus per time point ($n = 2$ fetuses). Data were log-transformed to remove heterogeneity of variance and then subjected to analysis of variance, followed by Duncan's multiple range test. Within each follicular stage, bars with no common superscripts are different: *a*, *b* $P < 0.05$).

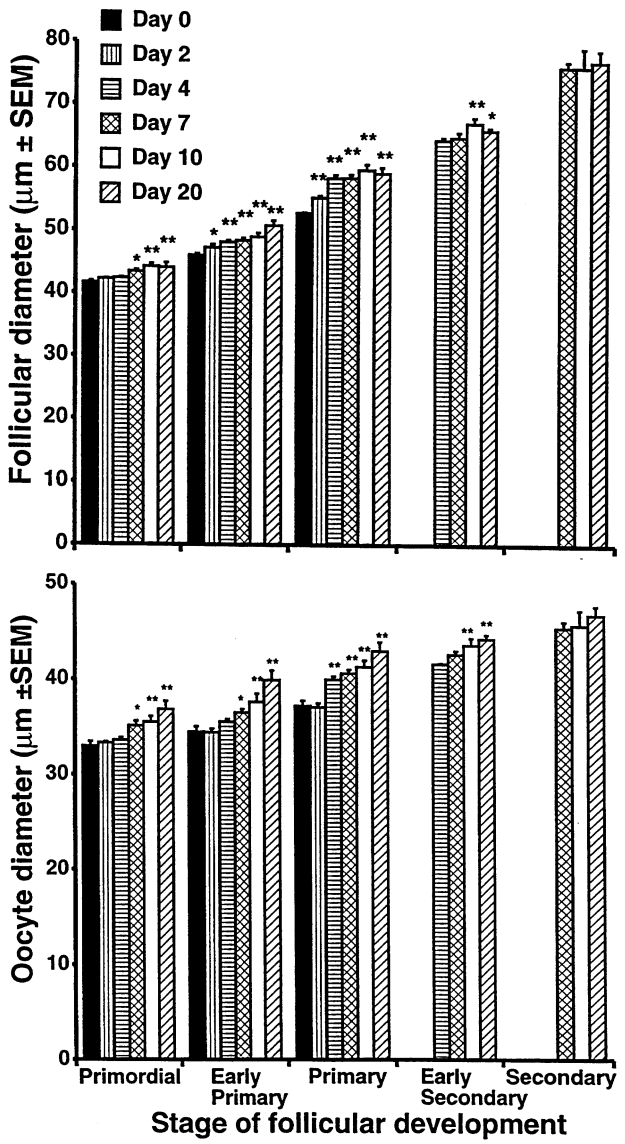

Fig. 4. Mean diameters (μm ± SEM) of healthy follicles and oocytes in pieces of baboon ovarian cortex after 0, 2, 4, 7, 10 or 20 days in culture in serum-free medium ($n = 3$ fetuses, with 200–252 primordial, 191–264 early primary, 209–236 primary, 84–94 early secondary and 29–40 secondary follicles/oocytes measured per fetus). Within each follicular stage, bars with asterisks are different from day 0 (*$P < 0.05$, **$P < 0.01$). Reprinted from Wandji et al., 1997 with permission.

When primordial follicles in whole, newborn mouse ovaries are activated in vitro in medium with 10% FBS they proceed to the secondary stage (i.e. preantral follicles with two or more layers of granulosa cells) within 8 days (the normal time in vivo) (Eppig and O'Brien, 1996). However, in our experiments with cortical cultures from fetal bovine and baboon ovaries very few follicles reached the secondary stage, even after 20 days in culture in the experiments with baboon cortex (Fig. 1). It is possible that the follicles require more time in vitro to develop to the secondary stage, but it seems more likely that either a specific hormone or growth factor is lacking in vitro or some general aspect of the environment (gas, nutrients, etc.) is not correctly formulated for optimum growth in vitro.

The type of culture medium has a significant influence on the survival and growth of follicles in cortical pieces from cattle and baboons. When we cultured bovine cortical pieces in 0, 2.5, 5, or 10% FBS with or without 0, 0.5X, or 1X ITS+, the usual precipitous decline in the number of primordial follicles occurred in all 12 culture media (Fortune et al., 1999). However, the media were not equally effective at promoting the survival and development of primary and secondary follicles, with half-strength (0.5X) ITS+ in combination with 5 or 10% FBS being the most effective. Nonetheless, even in these two media the numbers of secondary follicles were low. When baboon cortical pieces were cultured in medium containing 10% FBS, a curious phenomenon occurred. Many follicles were observed in which the oocyte had initiated growth and increased in diameter, but the surrounding granulosa cells were still flattened, as they are in primordial follicles (Wandji et al., 1997). This observation is interesting because it suggests that the primordial to primary follicle transition can be initiated separately in the two compartments of the follicle and this implies that more than one signal is involved in follicular activation.

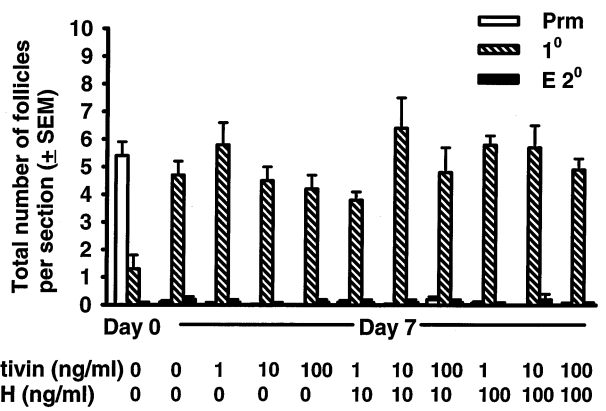

Fig. 5. Lack of effect of activin A or activin + FSH on numbers of follicles in pieces of bovine ovarian cortex after 7 days in culture (mean ± SEM, $n = 2$ fetuses). Pieces of fetal bovine ovarian cortex were isolated and cultured and numbers of primordial (Prm), primary (1°), and early secondary (E 2°) follicles determined as described previously (Wandji et al., 1996). Data were log-transformed to remove heterogeneity of variance and then subjected to analysis of variance.

Since bovine follicles bind FSH (Wandji et al., 1992b) and ovine follicles express messenger RNA for FSH receptor (Tisdall et al., 1995) as early as the primary stage of follicular development, we treated bovine ovarian cortical pieces in vitro with 0, 1, 10, or 100 ng/ml FSH and assessed follicular development after 7 or 14 days in culture (Fortune et al., 1998). There was no effect of any concentration of FSH on the percentages of primordial, primary, and secondary follicles after 7 or 14 days in vitro. This finding is consistent with results reported by Braw-Tal and Yossefi (1997) who found that addition of FSH (100 ng/ml) to cultures of adult bovine cortex did not increase the labelling index of granulosa cells at 2 days of culture. Mayerhofer et al. (1997) reported dramatic effects of FSH on the growth of follicles in neonatal rat ovaries, but only if the follicles were first treated with VIP or forskolin to increase cyclic AMP. Therefore, we treated bovine cortical pieces with VIP or forskolin for the first 8 h of culture and then cultured them for an additional 24 h with or without FSH. Although VIP and forskolin increased cyclic AMP in the culture medium dramatically, FSH had no effect on cAMP production during the subsequent 24 h (Fortune et al., 1999). These negative findings for FSH are consistent with a previous report that the FSH receptors on bovine primary follicles are not coupled to the adenylate cyclase second messenger system (Wandji et al., 1992a). The results are also consistent with the finding that primordial follicles that initiated growth in whole newborn mouse ovaries in vitro developed to the secondary stage by 8 days of culture, although the medium did not contain FSH (Eppig and O'Brien, 1996). Therefore, it appears that although FSH can accelerate the growth of the smallest preantral murine follicles, it is not required for such growth in vitro. In contrast, ruminant primary follicles do not seem to be responsive to FSH.

Since activin is reported to stimulate the growth of preantral follicles isolated from immature mice (Yokota et al., 1997; Liu et al., 1999), we treated bovine cortical pieces with graded doses of activin A (0, 1, 10, or 100 ng/ml) for 7 days. As expected, the number of primordial follicles was much lower on day 7 of culture compared with day 0, whereas the numbers of primary follicles had increased (Fig. 5). However, activin appeared to have no effect on the number of primary follicles and the number of secondary follicles remained low regardless of treatment. Negative data are always difficult to interpret since there may be a question of whether the right dose or time interval was employed or of whether the compound in question would be active if

given in combination with something else. When we treated cortical pieces with activin in combination with 10 or 100 ng/ml FSH, there was no effect on the number or distribution of follicles among the various categories (Fig. 5). Thus, there is no evidence so far that FSH or activin stimulate the growth of primary to secondary follicles in vitro, but the usual caveats about negative data apply.

6. Summary and conclusions

The primordial to primary follicle transition remains one of the least understood stages of follicular development. We have developed two experimental systems that may be used to study the activation of primordial follicles and to determine the requirements for further growth of follicles activated in vitro. In cultures of whole rodent ovaries or in CAM-grafted pieces of bovine or baboon ovarian cortex most follicles remain in the primordial stage. In contrast, primordial follicles in organ-cultured bovine or baboon ovarian cortex exhibit spontaneous and wholesale activation to the primary stage. In the future, comparison of these various models for studying early follicular development in mammals may provide clues about the mechanisms that stimulate follicles to join the growing pool and those that keep them arrested in the primordial stage. The ability to regulate the primordial to primary follicle transition is a prerequisite for use of the large reservoir of non-growing follicles to control fertility.

Acknowledgements

The contributions of Drs S.-A. Wandji, A.K. Voss, V. Srsen, J.J. Eppig, and P.W. Nathanielsz to the previously published results presented here (Figs. 1 and 4) are gratefully acknowledged. The unpublished data presented were generated through the support of the NIH (HD-35168 to JEF) and we are grateful for the contributions of C.A. Murphy and B.S. Hansen to those studies. The NIDDK provided the activin A and Taylor Packing (Wyalusing, PA) donated the fetal bovine ovaries used in these studies. Dr J.J. Eppig kindly read the manuscript.

References

Blandau, R.J., Warrick, E., Rumery, R.E., 1965. In vitro cultivation of fetal mouse ovaries. Fert. Steril. 16, 705–715.

Bodensteiner, K.J., Clay, C.M., Moeller, C.L., Sawyer, H.R., 1999. Molecular cloning of the ovine growth/differentiation factor-9 gene and expression of growth/differentiation factor-9 in ovine and bovine ovaries. Biol. Reprod. 60, 381–386.

Braw-Tal, R., Yossefi, S., 1997. Studies in vivo and in vitro on the initiation of follicle growth in the bovine ovary. J. Reprod. Fertil. 109, 165–171.

Dong, J., Albertini, D.F., Nishimori, K., Kumar, T.R., Lu, N., Matzuk, M.M., 1996. Growth differentiation factor-9 is required during early ovarian folliculogenesis. Nature 383, 531–535.

Eppig, J.J., O'Brien, M.J., 1996. Development in vitro of mouse oocytes from primordial follicles. Biol. Reprod. 54, 197–207.

Fortune, J.E., Kito, S., Byrd, D.D., 1999. Activation of primordial follicles in vitro. J. Reprod. Fertil. Suppl. 54, 439–448.

Fortune, J.E., Kito, S., Wandji, S.-A., Srsen, V., 1998. Activation of bovine and baboon primordial follicles in vitro. Theriogenology 49, 441–449.

Guraya, S.S., 1985. Primordial follicle. In: Biology of Ovarian Follicles in Mammals. Springer-Verlag, Berlin, pp. 3–14.

Henricson, B., Rajakoski, E., 1959. Studies of oocytogenesis in cattle. Cornell. Vet. 49, 494–503.

Hirshfield, A.N., 1991. Development of follicles in the mammalian ovary. Int. Rev. Cytol. 124, 43–101.

Hovatta, O., Silye, R., Abir, R., Krausz, T., Winston, R.M.L., 1997. Extracellular matrix improves survival of both stored and fresh human primordial and primary ovarian follicles in long-term culture. Hum. Reprod. 12, 1032–1036.

Liu, X., Andoh, K., Abe, Y., Kobayashi, J., Yamada, K., Mizunuma, H., Ibuki, Y., 1999. A comparative study on transforming growth factor-β and activin A for preantral follicles from adult, immature, and diethylstilbestrol-primed immature mice. Endocrinology 140, 2480–2485.

Manova, K., Huang, E.J., Angeles, M., De Leon, V., Sanchez, S., Pronovost, S.M., Besmer, P., Bachvarova, R.F., 1993. The expression pattern of the c-kit ligand in gonads of mice supports a role for the c-kit receptor in oocyte growth and in proliferation of spermatogonia. Dev. Biol. 157, 85–99.

Mayerhofer, A., Dissen, G.A., Costa, M.E., Ojeda, S.R., 1997. A role for neurotransmitters in early follicular development: induction of functional follicle-stimulating hormone receptors in newly formed follicles of the rat ovary. Endocrinology 138, 3320–3329.

Motro, B., Bernstein, A., 1993. Dynamic changes in ovarian c-kit and Steel expression during the estrous reproductive cycle. Dev. Dyn. 197, 69–79.

Parrott, J.A., Skinner, M.K., 1999. Kit-ligand/stem cell factor induces primordial follicle development and initiates folliculogenesis. Endocrinology 140, 4262–4271.

Rawles, M., 1952. Transplantation of normal embryonic tissues in the chick embryo in biological research. Ann. NY Acad. Sci. 55, 302.

Rudnick, D., 1944. Early history and mechanics of the chick blastoderm. Quart. Rev. Biol. 19, 187.

Russe, I., 1983. Oogenesis in cattle and sheep. Bibl. Anat. 24, 77–92.

Tisdall, D.J., Watanabe, K., Hudson, N.L., Smith, P., McNatty, K.P., 1995. FSH-receptor gene expression during ovarian follicle development in sheep. J. Mol. Endocrinol. 15, 273–281.

van Wagenen, G., Simpson, M.E., 1965. Embryology of the Ovary and Testis. *Homo sapiens* and *Macaca mulatta*. Yale University Press, New Haven.

van Wezel, I.L., Rodgers, R.J., 1996. Morphological characterization of bovine primordial follicles and their environment in vivo. Biol. Reprod. 55, 1003–1011.

Wandji, S.-A., Fortier, M.A., Sirard, M.-A., 1992a. Differential response to gonadotropins and prostaglandin E_2 in ovarian tissue during prenatal and postnatal development in cattle. Biol. Reprod. 46, 1034–1041.

Wandji, S.-A., Pelletier, G., Sirard, M.-A., 1992b. Ontogeny and cellular localization of ^{125}I-labeled insulin-like growth factor-I, ^{125}I-labeled follicle-stimulating hormone, and ^{125}I-labeled human chorionic gonadotropin binding sites in ovaries from bovine fetuses and neonatal calves. Biol. Reprod. 47, 814–822.

Wandji, S.-A., Srsen, V., Nathanielsz, P.W., Eppig, J.J., Fortune, J.E., 1997. Initiation of growth of baboon primordial follicles in vitro. Hum. Reprod. 12, 1993–2001.

Wandji, S.-A., Srsen, V., Voss, A.K., Eppig, J.J., Fortune, J.E., 1996. Initiation in vitro of growth of bovine primordial follicles. Biol. Reprod. 55, 942–948.

Yokota, H., Yamada, K., Liu, X., Kobayashi, J., Abe, Y., Mizunuma, H., Ibuki, Y., 1997. Paradoxical action of activin A on folliculogenesis in immature and adult mice. Endocrinology 138, 4572–4576.

Yoshida, H., Takakura, N., Kataoka, H., Kunisada, T., Okamura, H., Nishikawa, S.-I., 1997. Stepwise requirement of c-kit tyrosine kinase in mouse ovarian follicle development. Dev. Biol. 184, 122–137.