Immunolocalization of Growth, Inhibitory, and Proliferative Factors Involved in Initial Ovarian Folliculogenesis From Adult Common Squirrel Monkey (*Saimiri collinsi*)

Reproductive Sciences
2015, Vol. 22(1) 68-74
© The Author(s) 2014
Reprints and permission:
sagepub.com/journalsPermissions.nav
DOI: 10.1177/1933719114532842
rs.sagepub.com

S. R. R. A. Scalercio, MS^{1,2,3}, A. B. Brito, MS^{1,2}, S. F. S. Domingues, VMD, PhD^{1,2}, R. R. Santos, VMD, PhD^{1,2,4}, and C. A. Amorim, VMD, PhD⁵

Abstract

We performed an immunohistochemical (IHC) study to determine the follicular expression of growth differentiation factor 9 (GDF-9), anti-Müllerian hormone (AMH), Kit Ligand (KL), and c-Kit in squirrel monkey ovary. Ovarian tissue fragments from 4 squirrel monkeys were collected by laparotomy and processed for classical histology and IHC. Additionally, follicle development was assessed by Ki67 immunostaining to evaluate proliferative status of granulosa cells. A total of 4025 follicles were examined (1475 for classical histology and 2550 for immunohistochemistry). More than 80% of the evaluated follicles were morphologically normal. The GDF-9 protein was detectable in oocyte cytoplasm from primordial (100%), primary (99.1%), and secondary (100%) follicles. The AMH was not expressed in primordial follicles but just in few primary follicles (13.8%). On the other hand, it was highly expressed in granulosa cells from secondary follicles (67.9%). c-Kit, KL receptor, was found in the oolemma of primordial (100%), primary (100%), and secondary (100%) follicles. The KL expression was observed in oocytes and granulosa cells from primordial (94.9%), primary (91.6%) and secondary follicles (100%). Ki67 immunostaining was observed in granulosa cells from primary (5.7%) and secondary (54.8%) follicles but not in primordial follicles. In conclusion, we described the localization of GDF-9, KL, c-Kit, and Ki67 proteins and confirmed the presence of AMH protein in preantral follicles from squirrel monkey. Our results offer contribution for understanding of folliculogenesis in neotropical nonhuman primates. Moreover, these markers can be used to assess follicular viability and functionality after cryopreservation, transplantation, or in vitro culture of ovarian tissue.

Keywords

neotropical primate, immunohistochemistry, ovarian tissue, cell proliferation, preantral follicles

Introduction

Nonhuman primates (NHPs) have been largely used as model for biomedical research,¹ including human reproduction.² Regrettably, 40% of neotropical NHP species are listed as vulnerable, endangered, or critically endangered due to the continuous destruction of their natural habitats.³ However, information on key markers controlling ovarian folliculogenesis process in NHP is scarce. Besides, knowledge on the expression of strategic factors in different preantral follicles categories (primordial, primary, and secondary) supports the advances in reproductive technologies. For example, ovarian tissue cryopreservation is considered a success when follicles remain morphologically normal and are able to develop under in vitro culture conditions or after transplantation. The assessment of synchronic expression of growth factors and proper follicular activation and its subsequent development is decisive to determine the appropriateness of a technique. In this context, immunolocalization of growth, inhibitory, and proliferative

factors involved in ovarian folliculogenesis is essential for understanding mechanisms of ovarian follicle development regulation and to support analyses methods of captive breeding and preservation programs of endangered Neotropical NHP.

¹ Laboratory of Wild Animal Biology and Medicine, Federal University of Pará, Castanhal, Pará, Brazil

² Animal Sciences PhD Program, Federal University of Pará, Belém, Pará, Brazil

³ National Primate Centre, Secretary of Health Policy, Ministry of Health, Ananindeua, Pará, Brazil

⁴ Faculty of Veterinary Medicine, Utrecht University, Utrecht, the Netherlands

⁵ Pôle de Recherche en Gynécologie, Institut de Recherche Expérimentale et Clinique, Université Catholique de Louvain, Brussels, Belgium

Corresponding Author:

S. R. R. A. Scalercio, Laboratory of Wild Animal Biology and Medicine, Federal University of Pará, Castanhal, Rodovia BR-316km 7 s/n, Levilândia, CEP 67030-000, Ananindeua, Pará, Brazil.

Email: sarah.scalercio@cenp.org.br; sarahscalercio@gmail.com

Ovarian folliculogenesis is a complex and dynamic process in which the follicular development happens through the recruitment of the primordial follicles, with synergetic development of growing follicles until ovulation occurs. Cell proliferation, differentiation, and atresia associated with folliculogenesis is regulated by interaction of autocrine and paracrine signals, together with internal signaling pathways.^{4,5} Several intraovarian growth factors belonging to the superfamily of transforming growth factor β (TGF- β) are expressed in oocytes and granulosa cells from follicles at different stages of development, functioning as key regulators of ovarian folliculogenesis in mammals.⁶

In different NHP species, for instance, the TGF member growth differentiation factor 9 (GDF-9) is involved in ovarian follicular development, participating in recruitment of primordial follicles and proliferation and differentiation of granulosa cells.⁷ Moreover, GDF-9 appears to be present in different stages of ovarian follicle development in a species-specific manner. In cynomolgus monkey, GDF-9 protein expression was found in all stages of follicular development⁸; in rhesus monkeys, it was observed in preovulatory follicular cells⁹; in baboons, in primordial, primary, and secondary follicles.¹⁰ Anti-Müllerian hormone (AMH) is another member of the TGF- β superfamily playing an essential inhibitory role in the maintenance of the primordial follicles pool.² The AMH protein is expressed in granulosa cells from follicles at different stages of development in squirrel monkeys,² baboons,¹¹ bonnet monkey ovary,¹¹ and marmoset.¹² Another important growth factor identified in the ovarian follicle is the receptor tyrosine protein kinase Kit (c-Kit, CD117) and its ligand Kit Ligand (KL). The c-Kit/KL system plays an indispensable role in the follicular viability, primordial follicle recruitment, follicular development, and oocyte growth in mammal species.¹³ The activated c-Kit receptor participates in various signaling pathways including the regulation of cell proliferation, survival, migration, and cellular metabolism¹⁴ as well as participates in the activation of primordial follicles.¹⁵ In human ovaries, KL messenger RNA (mRNA) has been expressed at the primary follicular stage, and c-Kit protein can be present in oocytes and c-Kit mRNA in oocytes and granulosa cells from preovulatory follicles.^{16,17} In cynomolgus monkey, KL protein expression was found in granulosa cells, and c-Kit receptor at the oocyte cell surface from primordial follicles,⁸ and in capuchin monkeys, KL mRNA was quantified in ovarian tissue.¹⁸

Although species-specific differences are evident, studies on the dynamics of follicular development, and the relationship between growth factors, the recruitment of primordial follicles, and preantral follicles growth in Neotropical NHP are not available. Such information can help to optimize protocols of reproductive technology, serving as a tool to evaluate follicular viability and functionality. Hence, we aimed to immunolocalize the proteins GDF-9, AMH, c-Kit/KL, and Ki67 (a cell proliferation marker) at different stages of preantral follicular development in the ovaries from the Neotropical squirrel monkeys.

Materials and methods

Chemicals

Unless mentioned otherwise, the chemicals used in the present study were purchased from Sigma Chemical Co (St Louis, Missouri).

Animals

Our study was approved by the local ethical committee on Animal Research from the Evandro Chagas Institute (IEC/CEU no. 0009/2011) and by the Brazilian Institute for Wildlife and Environment (SISBIO/IBAMA no. 32632-1/2012) and followed the guidelines of the National Council of Control Animal Experimentation of Brazil. Four healthy and sexually mature squirrel monkey females (2.5–5 years old, weight range: 0.4–0.7 kg) born in captivity at National Primate Center, Pará, Brazil, were selected for our study. The females were kept indoors under a natural photoperiod and housed individually in cages (80 × 90 × 80 cm³) at the National Primate Center. Their daily diet consisted of fresh fruits, vegetables, and commercial monkey pellets (MEGAZOO P18, Betim, Brazil). Tap water was available ad libitum.

Ovarian Tissue Collection

All procedures were performed under general anesthesia. Each animal was anesthetized with ketamine hydrochloride (cetamine 10 mg/kg, intramuscularly [IM]; Köning S.A., Avellaneda, Argentina) and xylazine hydrochloride (anasedan 1 mg/kg, IM; Köning S.A.). Anesthesia was maintained with 2% halothane (Tanohalo; Cristália, São Paulo, Brazil). The females were placed in dorsal recumbence, and ovarian biopsies were collected by exploratory laparotomy. For this, a ventral midline skin incision was made to reach the ovaries as described by Domingues et al.¹⁹ One right ovary from each female was completely removed, cut into halves, and immediately fixed in paraformaldehyde for further histological and immunohistochemical (IHC) analyses. After surgery, the animals received antibiotics for 1 week (Chemitril; Chemitec, São Paulo, Brazil), and ibuprofen (Ketofen; Merial Saúde Animal Ltda, São Paulo, Brazil) was used to control the pain, in the 2 days following surgery.

Histological Analysis

After overnight fixation in 4% paraformaldehyde (pH 7.2) at 4°C, the ovarian fragments were dehydrated in ethanol, clarified in xylene, and embedded in paraffin wax. Serial sections (5 μ m) of ovarian tissue were cut and every 10th section was mounted on glass slides and stained with hematoxylin–eosin (Merck, Darmstadt, Germany). Preantral follicles with an oocyte surrounded by 1 layer of flattened granulosa cells were classified as primordial, while primary follicles presented 1 layer of cuboidal granulosa cells surrounding an oocyte and secondary follicles presented 2 or more layers of granulosa cells surrounding the oocyte.²⁰ Just follicles with a visible

Table 1. Antibodies Dilutions Used in the Present Study.

Antigen	Antibody	Dilution	Incubation
GDF-9	Rabbit polyclonal IgG	1:5000	4°C O/N
AMH	Mouse monoclonal IgG	1:30	4°C O/N
Ki67	Mouse monoclonal	1:50	4°C O/N
c-Kit	Rabbit polyclonal	1:1500	4°C O/N
KL	Mouse monoclonal IgG	1:25	4°C O/N

Abbreviations: AMH, anti-Müllerian hormone; GDF-9, growth differentiation factor 9; IgG, immunoglobulin G; KL, Kit Ligand; O/N = overnight.

nucleus were taken into account to avoid counting a follicle more than once. Histological sections were used to classify and count ovarian follicles that could be found in the following slides for IHC analyses, and extra sections were mounted in Superfrost Plus slides (Menzel-Glaser, Braunschweig, Germany) and submitted to IHC analysis. All sections were examined using a light microscope (Olympus, Tokyo, Japan) at a magnification of 400×.

Immunohistochemistry

Paraffin sections were deparaffinized with xylene (Sigma-Aldrich) and rehydrated in alcohol series. The endogenous peroxidase activity was blocked with 3% H₂O₂ diluted in methanol (for AMH and KL staining) or in deionized water (for GDF-9). The 0.3% H₂O₂ was diluted in deionized water and was used to block peroxidase activity during Ki67 and c-Kit staining. A demasking step was performed for 75 minutes at 98°C with citrate buffer and 20% Triton X100 before sections being subjected to antigen retrieval step. Antigen retrieval steps, antibody dilutions, and incubation conditions are summarized in Table 1. In brief, the slides were incubated for 60 minutes at room temperature with antirabbit for GDF-9 and c-Kit, antimouse for AMH, biotinylated rabbit antimouse for KL, and goat antimouse for Ki67 (Dako) secondary antibodies. For KL staining, streptavidin–HRP conjugate was also used, according to the streptavidin biotin method. Diaminobenzidine was used as a chromogen (SK4100; Vector Laboratories, Peterborough, United Kingdom). Hematoxylin was used for counterstaining, and rapid mounting medium to mount the slides (Entellan new; Merck). Negative controls consisted of the dilution solution without any primary antibody, and the following positive controls were used: hepatic carcinoma for GDF-9, human antral follicles for AMH, testicular germ cell tumor tissue for c-Kit, kidney carcinoma tissue for KL, and proliferative endometrium for Ki67. For quantitative analysis of GDF-9, AMH, KL, and c-Kit protein expression, primordial, primary, and secondary follicles were evaluated in all animals. Follicles were considered GDF-9-positive when the oocyte cytoplasm was immunostained; AMH-positive when at least 1 granulosa cell was immunostained; KL-positive when cytoplasm and cell membrane of oocytes and granulosa cells were immunostained; and for c-Kit when the oolemma of follicles showed stains. Ki67 staining was used to attest the growing status of follicles and to ensure that follicles classified as primordial were indeed

at resting stage. Follicles containing at least 1 Ki67-positive granulosa cell were considered as belonging to the growing pool (primary or secondary follicles).

Results

A total of 1475 preantral follicles was examined by classical histology, and the majority of them was morphologically normal (>80%). The percentages of follicles positively stained for AMH, GDF-9, c-Kit, KL, and Ki67 at the different developmental stages (primordial, primary, and secondary) are detailed in Table 2. All negative controls failed to show DAB-positive immunostaining, attesting the specificity of the markers.

Growth Differentiation Factor 9 Protein Expression

A total of 394 follicles (87 primordial, 235 primary, and 72 secondary follicles) were investigated, and the results of immunohistochemistry showed that GDF-9 protein was detectable by staining in oocytes cytoplasm from primordial (100%, 87 of 87), primary (99.1% ± 0.5%, 233 of 235), and secondary follicles (100%, 72 of 72). The staining intensity ranged from faint (primordial and primary follicles) to strong (secondary follicles). Granulosa cells also expressed GDF-9 (Figure 1A).

Anti-Müllerian Hormone Protein Expression

Anti-Müllerian Hormone staining varied according to the follicular stage of development. For this immunostaining, 382 follicles were counted (168 primordial, 109 primary, and 105 secondary). The granulosa cells from primary (13.8% ± 12.1%, 7 of 109) and secondary (67.9% ± 23.6%, 96 of 105) follicles were marked for AMH, and no primordial follicles showed staining for AMH (Figure 1B and C).

Cell Proliferation (Ki67 staining)

Cell proliferation was evaluated in 280 preantral follicles (133 primordial, 95 primary, and 52 secondary follicles) using Ki67 immunostaining. Some granulosa cells from primary (5.7% ± 5.7%, 5 of 95) and secondary (54.8% ± 21.8%, 25 of 52) follicles showed staining for Ki67, while all follicles classified as primordial showed no staining (Figure 1D and E).

c-Kit and KL Expression

Immunostaining was performed to localize c-Kit and KL protein expression. A total of 391 follicles were analyzed, and the oolemma of primordial (100%, 112 of 112), primary (100%, 242 of 242), and secondary (100%, 37 of 37) follicles were found strongly stained for c-Kit (Figure 2A and B). In KL staining, 1103 follicles were evaluated, and the KL protein was expressed in oocytes and granulosa cells of primordial (94.9% ± 2.4%, 762 of 814), primary (91.6% ± 4.2%, 217 of 243) and secondary (100%, 46 of 46) follicles. A faint staining was present in cytoplasm and

Table 2. Summary of the Expression of GDF-9, AMH, Kit Ligand, c-Kit, and Ki67 Protein in Ovarian Tissue of 4 Squirrel Monkeys.

Factors	Animal 1, n (% Stained)	Animal 2, n (% Stained)	Animal 3, n (% Stained)	Animal 4, n (% Stained)	Total, n (Mean % $\pm$ SEM)
GDF-9					
Primordial follicles	9 follicles	64 follicles	NFTS	14 follicles	87
Oocytes (cytoplasm)	(100)	(100)		(100)	(100 $\pm$ 0)
Primary follicles	95 follicles	75 follicles	NFTS	65 follicles	235
Oocytes (cytoplasm)	(100)	(98.7)		(98.5)	(99.1 $\pm$ 0.5)
Secondary follicles	10 follicles	48 follicles	NFTS	14 follicles	72
Oocytes (cytoplasm)	(100)	(100)		(100)	(100 $\pm$ 0)
AMH					
Primordial follicles	15 follicles	29 follicles	124 follicles	NFTS	168
Granulosa cells	(0)	(0)	(0)		(0 $\pm$ 0)
Primary follicles	73 follicles	19 follicles	12 follicles	5 follicles	109
Granulosa cells	(0)	(5.3)	(50)	(0)	(13.8 $\pm$ 12.1)
Secondary follicles	14 follicles	18 follicles	68 follicles	5 follicles	105
Granulosa cells	(71.4)	(100)	(100)	(0)	(67.9 $\pm$ 23.6)
Ki67					
Stromal cells	stained	stained	stained	stained	
Primordial follicles	42 follicles	38 follicles	5 follicles	48 follicles	133
Granulosa cells	(0)	(0)	(0)	(0)	(0 $\pm$ 0)
Primary follicles	39 follicles	29 follicles	5 follicles	22 follicles	95
Granulosa cells	(0)	(0)	(0)	(22.7)	(5.7 $\pm$ 5.7)
Secondary follicles	33 follicles	5 follicles	1 follicles	13 follicles	52
Granulosa cells	(42.4)	(0)	(100)	(76.9)	(54.8 $\pm$ 21.8)
c-Kit					
Primordial follicles	42 follicles	5 follicles	65 follicles	NFTS	112
Oocytes (oolema)	(100)	(100)	(100)		(100 $\pm$ 0)
Primary follicles	117 follicles	12 follicles	113 follicles	NFTS	242
Oocytes (oolema)	(100)	(100)	(100)		(100 $\pm$ 0)
Secondary follicles	21 follicles	2 follicles	14 follicles	NFTS	37
Oocytes (oolema)	(100)	(100)	(100)		(100 $\pm$ 0)
KL					
Primordial follicles	424 follicles	194 follicles	196 follicles	NFTS	814
Oocytes/granulosa cells	(90.3)	(98.4)	(95.9)		(94.9 $\pm$ 2.4)
Primary follicles	130 follicles	58 follicles	55 follicles	NFTS	243
Oocytes/granulosa cells	(83.8)	(98.2)	(92.7)		(91.6 $\pm$ 4.2)
Secondary follicles	16 follicles	6 follicles	24 follicles	NFTS	46
Oocytes/granulosa cells	(100)	(100)	(100)		(100 $\pm$ 0)

Abbreviations: AMH, anti-Müllerian hormone; GDF-9, growth differentiation factor 9; NFTS, no follicles in the tissue sections; KL, Kit Ligand; SEM, standard error of the mean.

cell membrane (Figure 2C and D). Interestingly, KL staining was also observed in cells from epithelial surface.

Discussion

Initial folliculogenesis in Neotropical NHP, genus *Saimiri*, has been poorly described. We know that primordial follicles can be found from birth until senile age, when this follicular population is depleted, leading to natural menopause.² A morphometric study of preantral follicles in senile monkeys²⁰ and a detailed study on the antral follicles development in this species were also performed.²¹

In the present study, we showed that ovarian follicles from squirrel monkey express GDF-9, c-Kit, and KL protein in all stages of follicular development (primordial, primary, and secondary). On the other hand, only AMH staining appears after

primary stage. To the best of our knowledge, except for 1 mRNA expression study,¹⁸ the expression of GDF-9 protein in ovarian tissue has not been investigated in Neotropical NHP as yet. The GDF-9 has a fundamental function in early folliculogenesis,²² being involved in the recruitment of primordial follicles and proliferation and differentiation of granulosa cells.⁷ The GDF-9 protein expression may vary according to the primate species. In squirrel monkeys, we observed that GDF-9 staining is present in oocytes and granulosa cells from follicles at all stages development. Similarly, in baboons, GDF-9 protein was also expressed in cytoplasm of oocytes from primordial, primary, and secondary and in the granulosa cells.¹⁰ In cynomolgus monkey, moderate GDF-9 expression was found only in oocytes from preantral and antral follicles, while granulosa cells were not stained.⁸ In rhesus monkeys, GDF-9 expression was observed in granulosa cells from preovulatory

Figure 1. Identification of the growth factors in oocytes and granulosa cells. A, Immunohistochemical staining for growth differentiation factor 9 (GDF-9) in primordial (asterisk), primary (arrow) and secondary follicles; (B) immunohistochemical staining for anti-Müllerian hormone (AMH), where primordial follicles are unstained, while (C) secondary follicles are positively stained; (D) immunohistochemical staining for the cell proliferation marker Ki67 showing unstained primordial follicles and (E) stained granulosa cells from secondary follicles in ovarian tissue from squirrel monkey.

follicles,⁹ and in humans, its expression was encountered in oocytes of primary and secondary follicles²³ but not from primordial follicles.²⁴ Although GDF-9 is a so-called oocyte-secreted factor,⁷ our findings corroborate with fact that different GDF-9 expression in primates is species specific when oocytes and granulosa cells from follicles at different developmental stages are evaluated. Several studies have demonstrated that GDF-9 exerts a critical role in oocyte viability,²⁵ follicular growth,²⁶ and thecal cell recruitment.²⁷ Attempts to understand such species-specific differences in GDF-9 expression and its role on folliculogenesis are, hence, necessary.

Anti-Müllerian hormone plays an essential inhibitory role to maintain the reserve of ovarian follicles throughout reproductive life span² by inhibiting primordial follicle activation.²⁸ Expression of AMH has also been reported in some NHP species. The AMH protein is expressed in granulosa cells from follicles at different stages of development in squirrel monkeys,² baboons,¹⁰ bonnet monkey,¹¹ and marmoset¹² ovaries. As previously reported, AMH is expressed in granulosa cells of growing follicles but not in primordial follicles from squirrel monkeys,² marmoset monkeys,¹² and human.²⁹ Differently, AMH expression in granulosa cells from ovarian follicles at all stages of development, including primordial follicles, has been reported in baboons.¹⁰

For the first time in a Neotropical NHP, the KL/c-Kit system has been identified in ovarian follicles. The interaction between KL and its receptor c-Kit is essential for the differentiation and development of ovarian follicles in different species.³⁰ In our study, c-Kit immunostaining in oolemma and KL protein were

found in oocytes and granulosa cells from preantral follicles. Similar findings were described in cryopreserved human ovarian follicles after xenotransplantation.²³ Brito et al¹⁸ evaluated KL mRNA expression in ovarian tissue from capuchin monkeys after in vitro culture, but in the study these later authors did not describe KL protein localization at different stages of follicular development. Abir et al³¹ reported the expression of KL and c-Kit proteins only in oocytes of primordial stages onward, in both fetal and adult ovaries. The c-Kit/KL system can assume important role in ovarian folliculogenesis in squirrel monkey, similar to other mammal species. In the communication between the oocytes and the granulosa cells, c-Kit/KL signaling may regulate the follicular viability, primordial follicle recruitment, follicular development, and oocyte growth in mammal species.¹³

Follicular growth activity was confirmed by proliferation of granulosa cells, as identified by Ki67 immunostaining at all stages of follicular development, except primordial follicles. Ki67 expression in primary and secondary follicles demonstrates that growing follicles are able to continue their development and may assume a healthy follicular status.

In this study, we have immunolocalized for the first time GDF-9, KL/c-Kit, and Ki67 proteins, as well as confirmed the expression of AMH protein in ovarian tissue from squirrel monkey. We believe that our results offer an important contribution for the understanding of folliculogenesis process in Neotropical monkeys. This knowledge is an important tool to assess follicular viability and functionality after cryopreservation, transplantation of ovarian tissue, and in vitro

Figure 2. Immunohistochemical staining for c-Kit and Kit Ligand in preantral follicles at different stages of development. A, Oolema of primordial (see arrow), primary and (B) secondary follicles stained for c-Kit. Dispersed Kit Ligand (KL) immunostaining was observed in oocytes and granulosa cells from (C) primordial and (D) primary (see arrow) and secondary follicles.

culture of early follicles. Furthermore, species-specific differences among primates open a window to future studies on follicular development and its mechanisms in human and NHPs.

Acknowledgments

The authors thank Dolores Gonzalez, Olivier van Kerk, and Osvaldo Leal dos Santos Filho for their technical assistance and the National Primate Center for the logistical support.

Authors' Note

Sarah R. R. A. Scalercio participated in Internship Program of Doctoral Sandwich Abroad in Université Catholique de Louvain, Brussels, Belgium.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship and/or publication of this article: Sarah R. R. A. Scalercio is a recipient of a grant from CAPES (Brazil).

References

1. Hau J, Schapiro SJ. Non-human primates in biomedical research. *Scand J Lab Anim Sci.* 2006;33(1):9-12.

2. Walker ML, Anderson DC, Herndon JG, Walker LC. Ovarian aging in squirrel monkeys (*Saimiri sciureus*). *Reproduction*. 2009;138(5):793-799.
3. IUCN Species Survival Commission, 2013. IUCN Red List Categories and Criteria, Version 2013.2. www.iucnredlist.org. Accessed January 17, 2014.
4. Fortune JE, Cushman RA, Wahl CM, Kito S. The primordial to primary follicle transition. *Mol Cell Endocrinol*. 2000;163(1-2): 53-60.
5. Suh CS, Sonntag B, Erickson GF. The ovarian life cycle: a contemporary view. *Rev Endocr Metab Disord*. 2002;3(1):5-12.
6. Knight PG, Glister C. TGF- β superfamily members and ovarian follicle development. *Reproduction*. 2006;132(2):191-206.
7. Paulini F, Melo EO. The role of oocyte-secreted factors GDF9 and BMP15 in follicular development and oogenesis. *Reprod Domest Anim*. 2011;46(2):354-361.
8. Gougeon A, Busso D. Morphologic and functional determinants of primordial and primary follicles in the monkey ovary. *Mol Cell Endocrinol*. 2000;163(1-2):33-41.
9. Duffy DM. Growth differentiation factor-9 is expressed by the primate follicle throughout the periovulatory interval. *Biol Reprod*. 2003;69(2):725-732.
10. Amorim AC, Jacobs S, Devireddy RV, et al. Successful vitrification and autografting of baboon (*Papio anubis*) ovarian tissue. *Hum Reprod*. 2013;28(8):2146-2156.
11. Modi D, Bhartiya D, Puri C. Developmental expression and cellular distribution of Müllerian inhibiting substance in the primate ovary. *Reproduction*. 2006;132(3):443-453.
12. Thomas FH, Telfer EE, Fraser HM. Expression of anti-Müllerian hormone protein during early follicular development in the primate ovary in vivo is influenced by suppression of gonadotropin secretion and inhibition of vascular endothelial growth factor. *Endocrinology*. 2007;148(5):2273-2281.
13. Driancourt MA, Reynaud K, Cortvrindt R, Smits J. Roles of KIT and KIT LIGAND in ovarian function. *Rev Reprod*. 2000;148(5): 143-152.
14. Cantley LC. The phosphoinositide 3-kinase pathway. *Science*. 2002;296(5573):1655-1657.
15. Adhikari D, Liu K. Molecular mechanisms underlying the activation of mammalian primordial follicles. *Endocr Rev*. 2009;30(5): 438-464.
16. Horie K, Fujita J, Takakura K, et al. The expression of c-Kit protein in human adult and fetal tissues. *Hum Reprod*. 1993; 8(11):1955-1962.
17. Tanikawa M, Harada T, Mitsunari M, Onohara Y, Iwabe T, Terakawa N. Expression of c-Kit messenger ribonucleic acid in human oocyte and presence of soluble c-Kit in follicular fluid. *J Clin Endocrinol Metab*. 1998;83(4):1239-1242.
18. Brito AB, Santos RR, Van den Hurk R, et al. Short-term culture of ovarian cortical strips from capuchin monkeys (*Sapajus apella*): a morphological, viability, and molecular study of preantral follicular development *in vitro*. *Reprod Sci*. 2013;20(8):990-997.
19. Domingues SFS, Caldas-Bussiere MC, Martins ND, Carvalho RA. Ultrasonographic imaging of the reproductive tract and surgical recovery of oocytes in *Cebus apella* (capuchin monkeys). *Theriogenology*. 2007;68(9):1251-1259.
20. Almeida DV, Santos RR, Scalercio SR, et al. Morphological and morphometrical characterization, and estimation of population of preantral ovarian follicles from senile common squirrel monkey (*Saimiri sciureus*). *Anim Reprod Sci*. 2012;134(3-4):210-215.
21. Dukelow WR. Reproductive cyclicity and breeding in the squirrel monkey. In: Rosenblum LA, Coe CL, eds. *Handbook of Squirrel Monkey Research*. New York, NY: New York, Inc; 1985:169-190.
22. Dong J, Albertini DF, Nishimori K, Kumar TR, Lu N, Matzuk MM. Growth differentiation factor-9 is required during early ovarian folliculogenesis. *Nature*. 1996;383(6600):531-535.
23. David A, Dolmans MM, Van Langendonck A, Donnez J, Amorim CA. Immunohistochemical localization of growth factors after cryopreservation and 3 weeks' xenotransplantation of human ovarian tissue. *Fertil Steril*. 2011;95(4):1241-1246.
24. Aaltonen J, Laitinen MP, Vuojolainen K, et al. Human growth differentiation factor 9 (GDF-9) and its novel homolog GDF-9B are expressed in oocytes during early folliculogenesis. *J Clin Endocrinol Metab*. 1999;84(8):2744-2750.
25. Carabatsos MJ, Elvin J, Matzuk MM, Albertini DF. Characterization of oocyte and follicle development in growth differentiation factor-9-deficient mice. *Dev Biol*. 1998;204(2):373-384.
26. Hreinsson JG, Scott JE, Rasmussen C, Swahn ML, Hsueh AJ, Hovatta O. Growth differentiation factor-9 promotes the growth, development, and survival of human ovarian follicles in organ culture. *J Clin Endocrinol Metab*. 2002;87(1):316-321.
27. Elvin JA, Yan C, Wang P, Nishimori K, Matzuk MM. Molecular characterization of the follicle defects in the growth differentiation factor-9-deficient ovary. *Mol Endocrinol*. 1999;13(6): 1018-1034.
28. Appt SE, Clarkson TB, Chen H, et al. Serum anti-Müllerian hormone predicts ovarian reserve in a monkey model. *Menopause*. 2009;16(3):597-601.
29. Weenen C, Laven JS, Von Bergh, et al. Anti-Müllerian hormone expression pattern in the human ovary: potential implications for initial and cyclic follicle recruitment. *Mol Hum Reprod*. 2004; 10(2):77-83.
30. Carlsson IB, Laitinen MP, Scott JE, et al. Kit ligand and c-Kit are expressed during early human ovarian follicular development and their interaction is required for the survival of follicles in long-term culture. *Reproduction*. 2006;131(2):641-649.
31. Abir R, Fisch B, Jin S, Barnnet M, Kessler-Icekson G, Ao A. Expression of stem cell factor and its receptor in human fetal and adult ovaries. *Fertil Steril*. 2004;82(suppl 3):1235-1243.