



## Ovarian follicle reserve: emerging concepts and applications

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### Abstract

This paper presents new concepts in the study of folliculogenesis and describes some of the current applications to reproductive biotechnology. The importance of better understanding this issue is addressed both for basic and applied research. After a brief review of the basic conceptions of the origin, formation, and growth of follicles according to established concepts, some controversial points, as the postnatal production of the follicles and the role of multiocyte follicles, are discussed. The importance of the ovarian follicular reserve is considered for fertility and reproductive parameters, as well as some questions about the presence of multiocyte follicles in adult ovaries. Finally, some future prospects are proposed.

**Keywords:** follicular population, folliculogenesis, multiocyte follicle, neo-folliculogenesis, preantral follicle.

### Introduction

The interest in both applied and basic issues relating to folliculogenesis has increased significantly in the recent years. Many points may clarify this increased interest. In commercial aspects, the industry of embryo production, most notably *in vitro* embryo production, has dramatically increased worldwide. Other biotechnologies, such as timed artificial insemination, cloning and transgenic animals have been widely used, due to their benefits on animal breeding or human health. However, many aspects in follicular physiology remain unknown, particularly the role of the ovarian follicle reserve and fertility in cattle. Regarding basic research, some new theories have been presented on the follicular pool, providing interesting debates about follicle origin and the mechanisms involved in their recruitment and growth. The purpose of this article is to summarize recent studies focusing on how the follicle reserve is related to the improvement of reproductive efficiency

### Basic concepts of folliculogenesis

In female mammals, folliculogenesis starts during fetal life (Fig. 1). First, primordial germ cells

migrate from the yolk sac to the primordial gonads. Initially by mitosis, these germ cells are multiplied and many groups of oogonia are established, interacting one to each other by cytoplasmic communications. After that, oogonia are surrounded by somatic cells, forming the cortical cords, which are precursors of primordial follicles. The oogonias differentiate into oocytes, which will form primordial follicles when associated with pregranulosa cells. Oocytes initiate meiotic division and arrest at the prophase of meiosis I, in diplotene stage. This interruption lasts until follicle recruitment, either in puberty or final period of the reproductive life (Soto-Suazo and Zorn, 2005; van den Hurk and Zhao, 2005).

### Antral follicles, number of oocytes, and embryo production

The impact of the size of the ovarian follicle reserve on applied aspects of reproduction, such as fertility and pregnancy rates has challenged the conventional concept of the ovary as a fairly static organ. There is high variability among individual animals in the numbers of preantral and antral follicles and oocytes in the ovaries of bovine females, and it seems that the breed and or subspecies may have a strong influence on that, especially when comparing *Bos taurus* vs. *Bos indicus* (Burns *et al.*, 2005; Ireland *et al.*, 2007; Santos *et al.*, 2012). However, there is repeatability in antral follicle count (AFC) within individuals regardless of age, breed, stage of lactation or season (Burns *et al.*, 2005). This repeatability was also observed on *Bos indicus-taurus* females (Santos *et al.*, 2012).

Extreme variation among donors in embryo production by *in vitro* and *in vivo* methods remains one of the problems in bovine embryo production. It is interesting to note that some donors present better results following MOET or IVF, independently of the number of follicles and oocytes (Pontes *et al.*, 2009). However, the most common situation is a higher production of embryos from those donors with higher number of follicles (Table 1).

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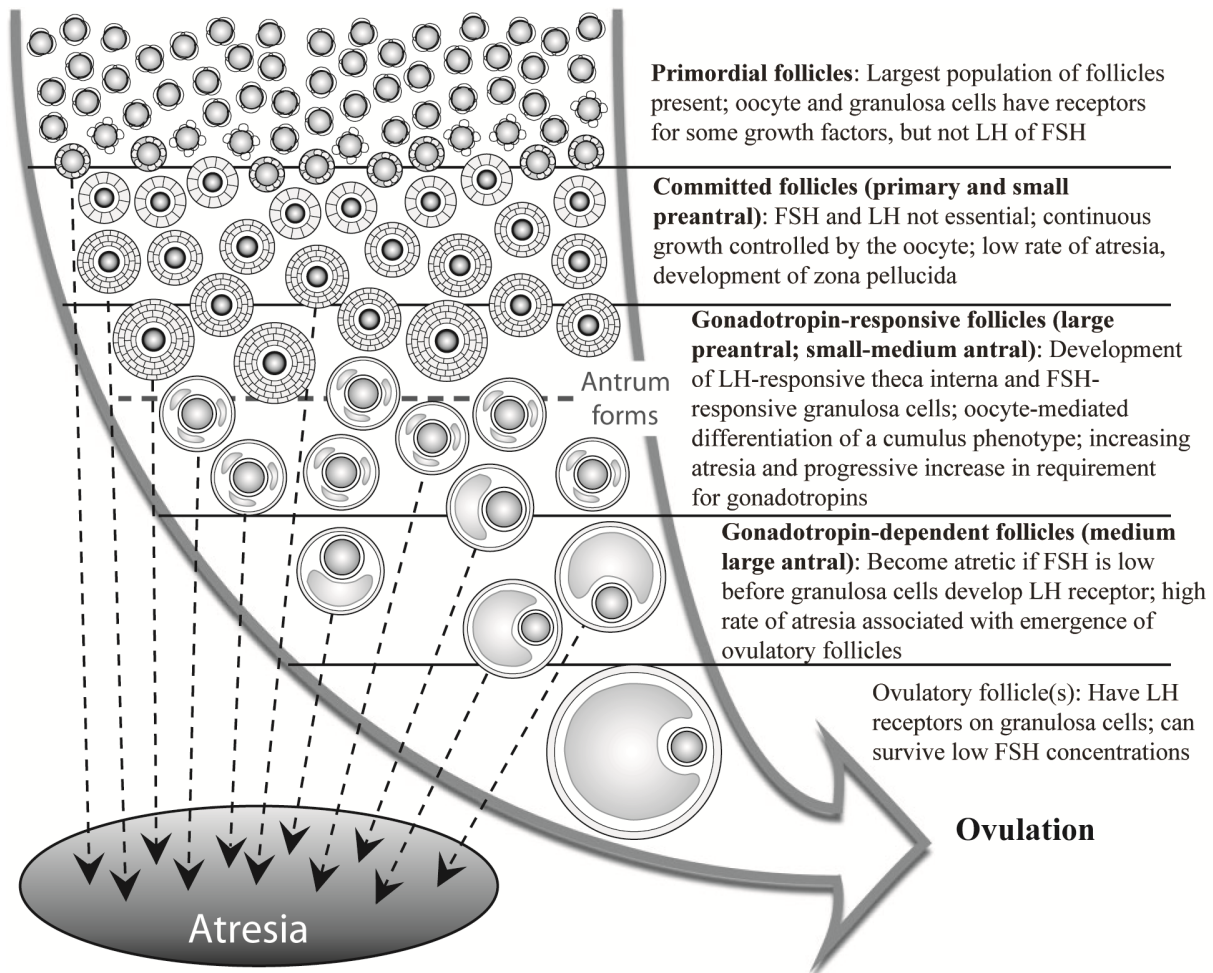


Figure 1. A model of folliculogenesis in ruminants. Adapted from Scaramuzzi *et al.* (2011).

Table 1. Variation in embryo production among 6 Nelore cows (I-VI), comparing *in vitro* (OPU/IVF) versus *in vivo* (MOET) procedures.

	Donors (I-VI)					
	I	II	III	IV	V	VI
Total no. OPU IVF cycles	5	5	4	4	5	5
Mean no. oocytes/collection	36.6	25.6	49	29.7	22.8	16
Mean no. viable oocytes/collection	32.2	23.4	45.2	26	19.6	14.4
Mean no. embryos/OPU IVF	15.6	10.4	24.1	10.3	6.8	3.8
Mean no. pregnancies/OPU IVF	4.8	2.8	9.25	4.3	2.2	1
Total no. MOET cycles	2	3	2	2	2	3
Mean no. embryos/collection	10	4.3	6.5	2	12.5	5.3
Mean no. pregnancies/collection	5.5	2	1	1.5	6.5	1.3

From Pontes *et al.* (2009).

In large scale commercial embryo production programs, individual animal variation in the number of follicles is very important (Table 2). The ordinary method of donor selection in this case consists in performing a pre-evaluation by ultrasound of the

ovaries, trying to identify those animals with higher numbers of antral follicles. It is important to emphasize that this selection, based on number of follicles, must be done after the evaluation of the genetic merit of the donors.



Table 2. Mean ( $\pm$  SD) for reproductive performance following OPU/IVP procedures (n = 656) performed in Nelore donors (n = 317), sorted according to oocyte collection (G1 = highest production and G4 = lowest production). Values given are per donor animal.

Group	No. cattle	No. viable oocytes	No. viable embryos	No. pregnancies on day 30	No. pregnancies on day 60
G1	n = 78	47.06 $\pm$ 1.6 <sup>a</sup>	15.06 $\pm$ 0.86 <sup>a</sup>	5.62 $\pm$ 0.54 <sup>a</sup>	5.52 $\pm$ 0.81 <sup>a</sup>
G2	n = 80	24.95 $\pm$ 0.33 <sup>b</sup>	9.17 $\pm$ 0.63 <sup>b</sup>	3.63 $\pm$ 0.36 <sup>b</sup>	3.32 $\pm$ 0.33 <sup>b</sup>
G3	n = 79	15.57 $\pm$ 0.26 <sup>c</sup>	6.00 $\pm$ 0.39 <sup>c</sup>	2.10 $\pm$ 0.21 <sup>c</sup>	1.92 $\pm$ 0.20 <sup>b</sup>
G4	n = 80	6.31 $\pm$ 0.38 <sup>d</sup>	2.42 $\pm$ 0.25 <sup>d</sup>	0.92 $\pm$ 0.13 <sup>d</sup>	0.85 $\pm$ 0.13 <sup>c</sup>
Total	n = 137	23.35 $\pm$ 0.72	8.13 $\pm$ 0.30	3.03 $\pm$ 0.15	2.91 $\pm$ 0.013

<sup>a,b,c,d</sup>Within a column, means without a common superscript differed (P < 0.05). From Pontes *et al.* (2011).

### Fertility and number of antral follicles

In recent years, low AFC in dairy cattle has been associated with some characteristics of low fertility, such as smaller ovaries, lower numbers of follicles and oocytes in the ovaries (Ireland *et al.*, 2008), lower odds of being pregnant in the end of the breeding season (Mossa *et al.*, 2012), reduced responsiveness to superovulation (Singh *et al.*, 2004; Ireland *et al.*, 2007), lower circulating concentrations of progesterone and anti-Müllerian hormone (AMH; Ireland *et al.*, 2008; Jimenez-Krassel *et al.*, 2009), reduced endometrial thickness from day 0 to day 6 of the estrous cycle (Jimenez-Krassel *et al.*, 2009), and higher amounts of cumulus cell markers for diminished oocyte quality (Ireland *et al.*, 2009).

Nutritional influences affect folliculogenesis at multiple levels (Scaramuzzi *et al.*, 2011). Increased energy supply is known to exert a stimulating effect at an ovarian level (Letelier *et al.*, 2008). Despite the fact that some dietary components like IGF-I or leptin are great stimulators of follicular growth (Muñoz-Gutiérrez *et al.*, 2005; Mihm and Evans, 2008), it is unlikely that there is a single metabolic mediator of nutritional influences on folliculogenesis.

In cattle suffering food restriction in the first trimester of pregnancy, the number of antral follicles of the fetus can be reduced up to 60%, although the weight of the calf at birth was not altered (Mossa *et al.*, 2013). Furthermore, in sheep variations in maternal diet can not only limit the growth of the fetus, but also inhibit the expression of genes related to the release of pituitary gonadotropins. The decrease in FSH and LH levels may in turn result in a reduction of the number of follicles

and delayed fetal ovarian development (Borwick *et al.*, 1997; da Silva *et al.*, 2002).

The health management of cattle on dairy farms influences long-term reproductive performance of the herd. Mammary gland infection is the major factor affecting somatic cell count (SCC) in milk. Studies suggest that offspring of cows with chronically high SCC during gestation have reduced anti-Müllerian hormone (AMH) concentrations. Lower AMH concentrations are indicative of a diminished size of the ovarian reserve and potential reduction in reproductive efficiency (Ireland *et al.*, 2010; Evans *et al.*, 2012).

### Population of antral follicles versus ovarian reserve

It is known that *Bos indicus* cattle have 3-4 times more antral follicles and number of oocytes than *Bos taurus* cattle (Pontes *et al.*, 2009, 2011). To test if the ovarian reserve could account for this difference, we counted preantral follicles in ovaries obtained at abattoirs from fetuses, heifers and cows (Nelore and Aberdeen Angus). There was no clear answer to explain why *Bos indicus* donors have more antral follicles and produce more oocytes than *B. taurus* females. Despite the high variation between groups and among females within the same group, there were no differences between the number of preantral follicles in the ovaries of fetuses, heifers or cows of Nelore versus *Bos taurus* females (Fig. 2). These results suggest that total follicle reserve is not the reason for the difference in oocyte yield (Silva-Santos *et al.*, 2011). We are currently investigating the rate of follicular atresia in Nelore cows and the possibility of it being lower in *indicus* than in *taurus* females.

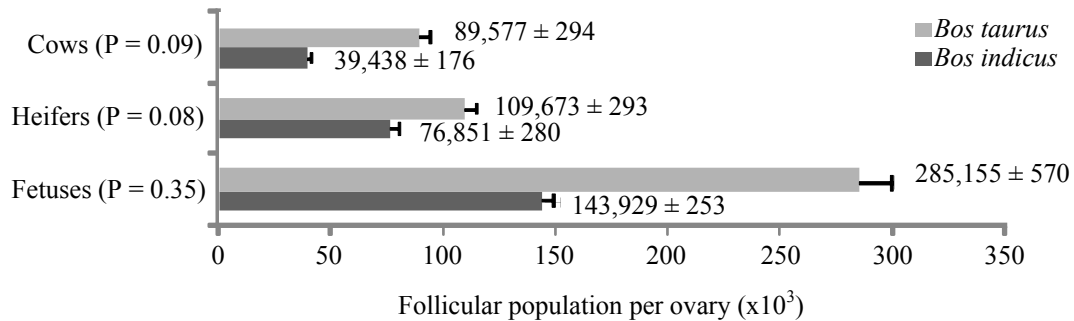


Figure 2. Average number of preantral follicles in the ovaries of *Bos indicus* (Nelore) and *Bos taurus* (Angus) females (mean ± SEM). From Silva-Santos *et al.* (2011).

### Molecular markers for the ovarian reserve

Inter-individual variability in oocyte production and in the response to exogenous ovarian stimulation remains a main limiting factor for embryo production in cattle. Thus, predictive tools for early selection of potentially high oocyte/embryo producing cattle are of critical importance in embryo programs. AMH, a member of the transforming growth factor- $\beta$  family produced by granulosa cells from healthy growing follicles, is currently recognized as a good marker of the ovarian reserve status and represents a good predictor of ovarian response to superstimulation (Monniaux *et al.*, 2010; Rico *et al.*, 2012). AMH concentration is correlated with the number of follicles recruited into the follicular waves during estrous cycles and is used as a biomarker of the follicle population in cattle. It was shown that cattle with high AFC (>25 follicles) presented higher circulating AMH concentrations compared to cattle with low AFC (<15 follicles;  $P < 0.01$ ) and a high correlation between the average AMH concentration and the average peak AFC was seen ( $r = 0.88$ ,  $P < 0.001$ ; Ireland *et al.*, 2008). Moreover, AMH concentrations vary minimally during oestrous cycles of beef and dairy cows (Ireland *et al.*, 2008; Rico *et al.*, 2009) and during menstrual cycles in women (Hehenkamp *et al.*, 2006; La Marca *et al.*, 2006), and has the same level of accuracy and clinical value as AFC to predict the response in assisted reproduction therapy (ART; Hendriks *et al.*, 2005, 2007; Broer *et al.*, 2009).

Considering the relationship of AMH with embryo production and antral follicle count, it is interesting to note that a single ultrasound evaluation presents a good picture of the number of antral follicles recruited per wave in that animal, in all reproductive cycles. This way, we can think about taking the ovary scanning as a possible alternative to predict the AMH level.

### The neo-folliculogenesis debate

For more than a century, it has been generally accepted that oocytes cannot be renewed in postnatal life. According to this concept, the number of oocytes is permanently defined in fetal ovaries (Zuckerman, 1951).

Nevertheless, in the last decade, this assumption has been in discussion. A group reported the presence of specific markers of meiosis in the ovaries of adult mouse females (Johnson *et al.*, 2004). Giving the current concept, such event should only take place during the fetal phase. A year later, female mice were subjected to chemical sterilization, with reports of the absence of follicles after the treatment. The same animals received a transfusion of bone marrow and peripheral blood and, a week later, viable follicles were identified in the ovaries (Johnson *et al.*, 2005).

The hypothesis of Johnson *et al.* (2004, 2005) has been strongly debated and some aspects of the experiments were heavily criticized by other researchers. Eggan *et al.* (2006) investigated the follicular renewal from cells spread through the blood in parabiotic mice (animals with shared bloodstream). Since one of the females was transgenic for a fluorescent protein, it was assumed that fluorescent oocytes should be found in the ovaries of the other female. However, even after repeated trials, the results have been contradictory to the neo-folliculogenesis theory.

Indirectly, in accordance with the theory of postnatal follicular renewal, Dyce *et al.* (2006) isolated follicle-like structures from porcine skin-derived fetal stem cells. These structures were capable of producing steroid hormones and were responsive to gonadotropins, in addition to producing embryo-like structures by parthenogenesis. Conversely, Liu *et al.* (2007) searched adult human ovaries for the presence of key genes involved in meiosis. No evidence of the occurrence of meiosis was found, contradicting the hypothesis of follicular renewal.

More recently, Kerr *et al.* (2012) monitored the number of primordial follicles throughout postnatal life and following depletion of the primordial follicle reserve, reported no indication of follicular renewal. Accordingly, Zhang *et al.* (2012) traced the development of female germline cell lineage and found no mitotically active female germline progenitors in postnatal ovaries. On the other hand, somatic cell generated from differentiating embryonic stem cells (ESCs) expressed specific granulosa cell markers. When

injected into neonatal mouse ovaries, these cells became incorporated within the granulosa cell layer of immature follicles and were able to synthesize steroids and respond to FSH (Woods *et al.*, 2013). Showing new data in the neo-folliculogenesis theory, Tilly's group described a protocol for isolation of female germline stem cells from adult ovarian tissue, along with cultivation and characterization of these cells before and after ex-vivo expansion (Woods and Tilly, 2013).

Independently of the individual opinion on the matter, we consider that the discussion is valuable, since new information has been added on the knowledge of follicular reserve.

### Multioocyte follicles

Follicles containing two or more oocytes have been described in the ovaries of adult females of several mammalian species (reviewed by Silva-Santos and

Seneda, 2011). These structures have been called multioocyte follicles (Fig. 3) and their contribution to ovulation and fertility in adult females is not currently known. Follicles with more than one oocyte have been well documented during fetal development. In this period, the ovarian cords are formed, which are tube-like structures containing germ cells surrounded by pregranulosa cells (Juengel *et al.*, 2002). However, the presence of these structures in adult ovaries is an intriguing physiologic phenomenon. It remains to be determined if these multioocyte follicles are simply remaining structures of the fetal phase or if they could be active and have a roll in an unknown pattern of follicle development in adult females. The presence of multioocyte follicles in *Bos indicus* females is particularly intriguing, considering the higher number of antral follicles and the same number of preantral follicles when comparing *Bos taurus* and *Bos indicus* ovaries (Silva-Santos *et al.*, 2011).

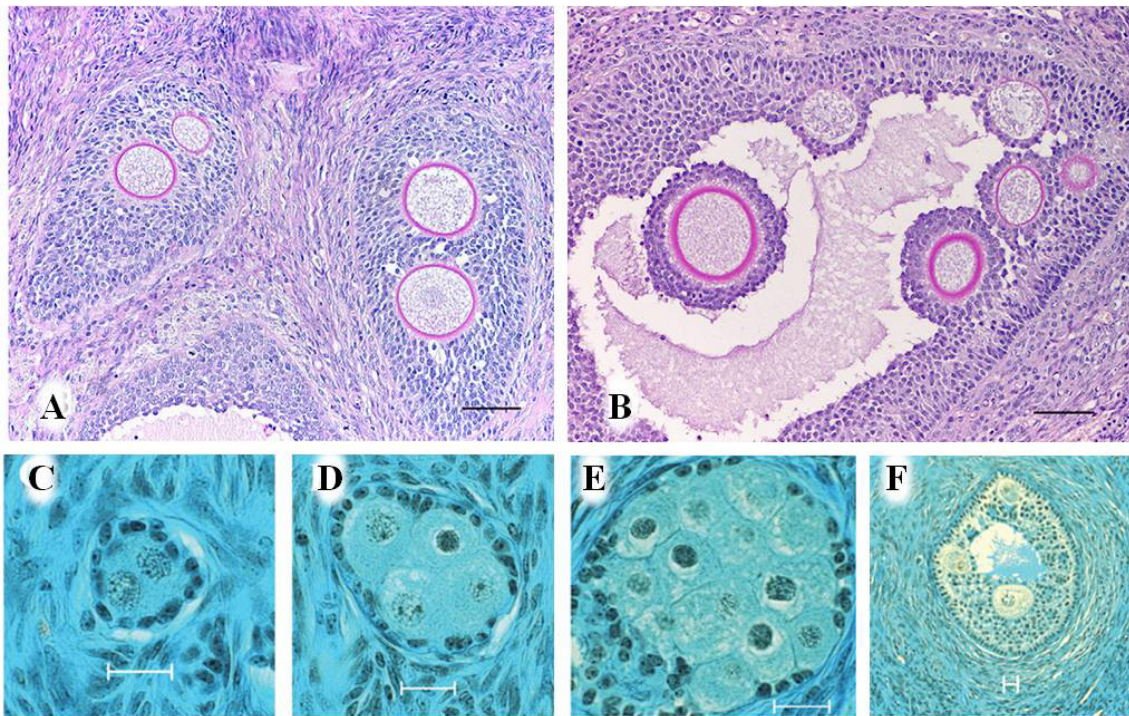


Figure 3. Preantral and antral multioocyte follicles in mature bitch (A-B) and heifer (C-F) ovaries. Multioocyte follicle at the secondary stage of development with two oocytes in each follicle (A), at the antral stage with six oocytes (B), at the primary stage with two (C), four (D) and 13 oocytes (E), and at the antral stage with three oocytes (F). Scale bars: 70  $\mu$ m. Adapted from Ireland *et al.* (2008); Payan-Carreira and Pires (2008).

### Conclusions and future remarks

As briefly described, folliculogenesis remains as a universe to be explored. Despite the extraordinary progress we have seen in the last years, there are several gaps to be filled, especially in the preantral phase. Neo-oogenesis can still be considered as a hypothesis. However, it seems reasonable to discuss that the follicle

formation may have new concepts not understood so far. For instance, we need to improve our knowledge about the multioocyte follicles and the follicular cords in adult females. Also, the number of oocytes obtained from *indicus* cows remains unclear at this moment, since the number of preantral follicles in Zebu females is similar to the amount observed in *taurus* animals.

Cattle can be selected based on counting antral



follicles using ultrasonography. Its use in the field is stimulating, since ultrasonography is an easy and fast tool that can be applied and that should not alter management conditions. Therefore, more oocytes/embryos per donor may be obtained. However, the genetic impact of selecting cattle with high AFC remains to be considered, especially taking into account the large number of descendants that can be produced by IVF from a single donor.

### References

- Borwick SC, Rhind SM, McMillen SR, Racey PA.** 1997. Effect of undernutrition of ewes from the time of mating on fetal ovarian development in mid-gestation. *Reprod Fertil Dev*, 9:711-715.
- Broer SL, Mol BWJ, Hendriks D, Broekmans FJM.** 2009. The role of antimullerian hormone in prediction of outcome after IVF: comparison with the antral follicle count. *Fertil Steril*, 91:705-714.
- Burns DS, Jimenez-Krassel F, Ireland JLH, Knight PG, Ireland JJ.** 2005. Numbers of antral follicles during follicular waves in cattle: evidence for high variation among animals, very high repeatability in individuals, and an inverse association with serum follicle-stimulating hormone concentrations. *Biol Reprod*, 73:53-62.
- Da Silva P, Aitken RP, Rhind SM, Racey PA, Wallace JM.** 2002. Impact of maternal nutrition during pregnancy on pituitary gonadotrophin gene expression and ovarian development in growth restricted and normally grown late gestation sheep fetuses. *Reproduction*, 123:769-777.
- Dyce PW, Lihua W, Li J.** 2006. In vitro germline potential of stem cells derived from fetal porcine skin. *Nat Cell Biol*, 8:384-390.
- Eggan K, Jurga S, Gosden R, Min IM, Wagers AJ.** 2006. Ovulated oocytes in adult mice derive from non-circulating germ cells. *Nature*, 441:1109-1114.
- Evans ACO, Mossa F, Walsh SW, Scheetz D, Jimenez-Krassel F, Ireland JLH, Smith GW, Ireland JJ.** 2012. Effects of maternal environment during gestation on ovarian folliculogenesis and consequences for fertility in bovine offspring. *Reprod Domest Anim*, 47:31-37.
- Hehenkamp WJK, Looman CWN, Themmen APN, De Jong FH, Te Velde ER, Broekmans FJM.** 2006. Anti-Mullerian hormone levels in the spontaneous menstrual cycle do not show substantial fluctuation. *J Clin Endocrinol Metab*, 91:4057-4063.
- Hendriks DJ, Mol BW, Bancsi LF, Te Velde ER, Broekmans FJ.** 2005. Antral follicle count in the prediction of poor ovarian response and pregnancy after in vitro fertilization: a meta-analysis and comparison with basal follicle-stimulating hormone level. *Fertil Steril*, 83:291-301.
- Hendriks DJ, Kwee J, Mol BW, Te Velde ER, Broekmans FJ.** 2007. Ultrasonography as a tool for the prediction of outcome in IVF patients: a comparative meta-analysis of ovarian volume and antral follicle count. *Fertil Steril*, 87:764-775.
- Ireland JJ, Ward F, Jimenez-Krassel F, Ireland JHL, Smith GW, Lonergan P, Evans ACO.** 2007. Follicle numbers are highly repeatable within individual animals but are inversely correlated with FSH concentrations and the proportion of good-quality embryos after ovarian stimulation in cattle. *Hum Reprod*, 22:1687-1695.
- Ireland JLH, Scheetz D, Jimenez-Krassel F, Themmen APN, Ward F, Lonergan P, Smith GW, Perez GI, Evans ACO, Ireland JJ.** 2008. Antral follicle count reliably predicts number of morphologically healthy oocytes and follicles in ovaries of young adult cattle. *Biol Reprod*, 79:1219-1225.
- Ireland JJ, Zielak AE, Jimenez-Krassel F, Folger J, Bettogowda A, Scheetz D, Walsh S, Mossa F, Knight PG, Smith GW, Lonergan P, Evans ACO.** 2009. Variation in the ovarian reserve is linked to alterations in intrafollicular oestradiol production and ovarian biomarkers of follicular differentiation and oocyte quality in cattle. *Biol Reprod*, 80:954-964.
- Ireland JJ, Scheetz D, Jimenez-Krassel F, Folger JK, Smith GW, Mossa F, Evans ACO.** 2010. Evidence that mammary gland infection/injury during pregnancy in dairy cows may have a negative impact on size of the ovarian reserve in their daughters. *Biol Reprod*, 83:277. (abstract).
- Jimenez-Krassel F, Folger J, Ireland JLH, Smith GW, Hou X, Davis JS, Lonergan P, Evans ACO, Ireland JJ.** 2009. Evidence that high variation in ovarian reserves of healthy young adults has a negative impact on the corpus luteum and endometrium during reproductive cycles of single-ovulating species. *Biol Reprod*, 80:1272-1281.
- Johnson J, Canning J, Kaneko T, Pru JK, Tilly JL.** 2004. Germline stem cells and follicular renewal in the postnatal mammalian ovary. *Nature*, 428:145-150.
- Johnson J, Bagley J, Skaznik-Wikiel M, Lee HJ, Adams GB, Niikura Y, Tschudy KS, Tilli JC, Cortes ML, Forkert R, Spitzer T, Iacomini J, Scadden DT, Tilly JL.** 2005. Oocyte generation in adult mammalian ovaries by putative germ cells in bone marrow and peripheral blood. *Cell*, 122:303-315.
- Juengel JL, Sawyer HR, Smith PR, Quirke LD, Heath DA, Lun S, Wakefield SJ, McNatty KP.** 2002. Origins of follicular cells and ontogeny of steroidogenesis in ovine fetal ovaries. *Mol Cell Endocrinol*, 191:1-10.
- Kerr JB, Brogan L, Myers M, Hutt KJ, Mladenovska T, Ricardo S, Hamza K, Scott CL, Strasser A, Findlay JK.** 2012. The primordial follicle reserve is not renewed after chemical or  $\gamma$ -irradiation mediated depletion. *Reproduction*, 143:469-476.
- La Marca A, Stabile G, Arsenio AC, Volpe A.** 2006. Serum anti-Mullerian hormone throughout the human menstrual cycle. *Hum Reprod*, 21:3103-3107.



- Letelier C, Mallo F, Encinas T, Ros JM, Gonzalez-Bulnes A.** 2008. Glucogenic supply increases ovulation rate by modifying follicle recruitment and subsequent development of preovulatory follicles without effects on ghrelin secretion. *Reproduction*, 136:65-72.
- Liu Y, Wu C, Lyu Q, Yang D, Albertini DF, Keefe DL, Liu L.** 2007. Germline stem cells and neo-oogenesis in the adult human ovary. *Dev Biol*, 306:112-120.
- Mihm M, Evans AC.** 2008. Mechanisms for dominant follicle selection in monovulatory species: a comparison of morphological, endocrine and intraovarian events in cows, mares and women. *Reprod Domest Anim*, 43:48-56.
- Monniaux D, Barbey S, Rico C, Fabre S, Gallard Y, Larroque H.** 2010. Anti-Müllerian hormone: a predictive marker of embryo production in cattle. *Reprod Fertil Dev*, 22:1083-1091.
- Mossa F, Walsh SW, Butler ST, Berry DP, Carter F, Lonergan P, Smith GW, Ireland JJ, Evans AC.** 2012. Low numbers of ovarian follicles  $\geq 3$  mm in diameter are associated with low fertility in dairy cows. *J Dairy Sci*, 95:2355-2361.
- Mossa F, Carter F, Walsh SW, Kenny DA, Smith GW, Ireland JLH, Hildebrandt TB, Lonergan P, Ireland JJ, Evans ACO.** 2013. Maternal undernutrition in cows impairs ovarian and cardiovascular systems in their offspring. *Biol Reprod*, 88:92, 1-9.
- Muñoz-Gutiérrez M, Finlay P, Adam CL, Wax G, Campbell BK, Kendall NR, Khalid M, Forsberg M, Scaramuzzi RJ.** 2005. The effect of leptin on folliculogenesis and the follicular expression of mRNA encoding aromatase, IGF-IR, IGFBP-2, IGFBP-4, IGFBP-5, leptin receptor and leptin in ewes. *Reproduction*, 130:869-881.
- Payan-Carreira R, Pires MA.** 2008. Multiocyte follicles in domestic dogs: a survey of frequency of occurrence. *Theriogenology*, 69:977-982.
- Pontes JHF, Nonato-Junior I, Sanches BV, Ereno-Junior JC, Uvo S, Barreiros TRR, Oliveira JA, Hasler JF, Seneda MM.** 2009. Comparison of embryo yield and pregnancy rate between in vivo and in vitro methods in the same Nelore (*Bos indicus*) donor cows. *Theriogenology*, 71:690-697.
- Pontes JHF, Melo-Sterza FA, Basso AC, Ferreira CR, Sanches BV, Rubin KCP, Seneda MM.** 2011. Ovum pick up, in vitro embryo production, and pregnancy rates from a large-scale commercial program using Nelore cattle (*Bos indicus*) donors. *Theriogenology*, 75:1640-1646.
- Rico C, Fabre S, Médigue C, Di Clemente N, Clément F, Bontoux M, Touzé JL, Dupont M, Briant E, Rémy B, Beckers JF, Monniaux D.** 2009. Antimüllerian hormone is an endocrine marker of ovarian gonadotropin responsive follicles and can help to predict superovulatory responses in the cow. *Biol Reprod*, 80:50-59.
- Rico C, Drouilhet L, Salvetti P, Dalbiès-Tran R, Jarrier P, Touzé JL, Pillet E, Ponsart C, Fabre S, Monniaux D.** 2012. Determination of anti-Müllerian hormone concentrations in blood as a tool to select Holstein donor cows for embryo production: from the laboratory to the farm. *Reprod Fertil Dev*, 24:932-944.
- Santos GMG, Silva-Santos KC, Siloto LS, Morotti F, Marcantonio TN, Marinho LSR, Thasmo RLO, Koetz JR C, Cintra DML, Seneda MM.** 2012. Dinâmica folicular em fêmeas bovinas de alta, média e baixa contagem de folículos antrais: resultados preliminares. *Acta Sci Vet*, 40:422. (abstract).
- Scaramuzzi RJ, Baird DT, Campbell BK, Driancourt MA, Dupont J, Fortune JE, Gilchrist RB, Martin GB, McNatty KP, McNeilly AS, Monget P, Monniaux D, Viñoles C, Webb R.** 2011. Regulation of folliculogenesis and the determination of ovulation rate in ruminants. *Reprod Fertil Dev*, 23:444-467.
- Silva-Santos KC, Santos GMG, Siloto LS, Hertel MF, Andrade ER, Rubin MIB, Sturion L, Sterza FAM, Seneda MM.** 2011. Estimate of the population of preantral follicles in the ovaries of *Bos taurus indicus* and *Bos taurus taurus* females. *Theriogenology*, 76:1051-1057.
- Silva-Santos KC, Seneda MM.** 2011. Multiocyte follicles in adult mammalian ovaries. *Anim Reprod*, 8:58-67.
- Singh J, Dominguez M, Jaiswal R, Adams GP.** 2004. A simple ultrasound test to predict the superstimulatory response in cattle. *Theriogenology*, 62:227-243.
- Soto-Suazo M, Zorn TM.** 2005. Primordial germ cells migration: morphological and molecular aspects. *Anim Reprod*, 3:147-60.
- van den Hurk R, Zhao J.** 2005. Formation of mammalian oocytes and their growth, differentiation and maturation within ovarian follicles. *Theriogenology*, 63:1717-1751.
- Woods DC, Tilly JL.** 2013. Isolation, characterization and propagation of mitotically active germ cells from adult mouse and human ovaries. *Nature Protocols*, 8:966-988.
- Woods DC, White YAR, Niikura Y, Kiatpongson S, Lee H, Tilly JL.** 2013. Embryonic stem cell-derived granulosa cells participate in ovarian follicle formation in vitro and in vivo. *Reprod Sci*, 20:524-535.
- Zhang H, Zheng W, Shen Y, Adhikari D, Ueno H, Liu K.** 2012. Experimental evidence showing that no mitotically active female germline progenitors exist in postnatal mouse ovaries. *Proc Natl Acad Sci USA*, 109:12580-12585.
- Zuckerman S.** 1951. The number of oocytes in the mature ovary. *Recent Prog Horm Res*, 6:63-108.