www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; SP-12(8): 1826-1830

www.thepharmajournal.com
Received: 18-05-2023
Accepted: 30-07-2023

Preeti
Department of Veterinary Gynaecology and Obstetrics, GADVASU, Ludhiana, Punjab, India

Anjali
Department of Veterinary Anatomy, KVAFSU, Bidar, Karnataka, India

Akshata Patil
Ph.D., Scholar, Department of Animal Genetics and Breeding, NDRI, Haryana, India

Rajeshwari
Ph.D., Scholar, Department of Veterinary Parasitology, CoVAS Mannuthy, Kerala, India

Abhishek Bhardwaj
Ph.D., Scholars, Department of Veterinary Gynaecology and Obstetrics, GADVASU, Ludhiana, Punjab, India

Jayanthi KV
Assistant Professor, Department of Animal Genetics and Breeding, Hassan, Karnataka, India

Corresponding Author:
Preeti
Department of Veterinary Gynaecology and Obstetrics, GADVASU, Ludhiana, Punjab, India

Factors affecting *in vitro* maturation of bovine oocytes

Preeti, Anjali, Akshata Patil, Rajeshwari, Abhishek Bhardwaj and Jayanthi KV

Abstract
Reproductive biotechnologies and genetic improvement programmes, have emerged as a strategy for increasing animal production. Ovum pick-up (OPU) has evolved as popular reproductive biotechnology in cattle from super stimulation and boosts maternal contribution to genetic improvement. OPU leads to significant genetic selection, and has decreased the time interval between generations and increased herd productivity. Following *in vitro* maturation (IVM), the majority of oocytes (90%) will enter metaphase II (M-II) and extrude the first polar body; about 80 percent will progress through fertilization and cleave to the two-cell stage. However, only 30–40 percent of embryos will ever develop into blastocysts. This would imply that the lengthiest phase of the *in vitro* embryo production process, the post-fertilization phase, is the key factor affecting blastocyst output. This is incorrect, as the percentage of immature oocytes those form the blastocyst is determined by events that occurred earlier along the developmental axis. The quality of blastocysts that do form is greatly influenced by the post-fertilization culture period, with *in vitro* created blastocysts continuously being of lower quality than their *in vivo* produced counterparts. The task is to alter the settings in our *in vitro* cultures for producing embryos in an effort to replicate those that naturally occur *in vivo* and so enhance blastocyst quality.

Keywords: Ovum pick up, *in vitro* maturation, oocytes, bovine

Introduction
The OPU-IVF process becomes even more important due to the existing ban on cow slaughter on the Indian subcontinent. OPU-IVF is a significant assisted reproductive approach for generating a more offspring from high-quality cattle, permitting increased selection intensity and shorter generation intervals the use of young heifers. In the cattle breeding and embryo transfer industries, the oocyte retrieval method and *in vitro* embryo production (IVEP) have proven to be useful innovations. Variability in the quality and developmental competence of retrieved oocytes and their developmental potency rests a foremost concern (Duszewska & Reklewski, 2000) [11].

Oocyte competence Cytoplasmic and nuclear maturation
Oocyte maturation cycle requires nuclear and cytoplasmic maturation. Between 21 to 24 hours following the start of IVM, the majority of buffalo oocytes reach nuclear maturity. Oocyte maturation conditions in synchronous to the sperm is crucial for successful fertilization. Sperm-oocyte co-incubation of 16 hours is essential for maximal blastocyst yields, while cleavage and blastocyst rates linearly decline as *in vitro* maturation time surges from 18 to 30 h (Gasparrini *et al*., 2008) [13]. Oocytes in follicles are in a dormant germinal vesicle (GV-prophase-I of meiosis) stage when punctured by OPU. Oocytes retrieved by OPU or slaughterhouse matured are immature oocytes resting at prophase stage after puberty is attained, the first meiotic division is completed under physiological conditions. Germinal vesicle breakdown (GVBD) is a breakthrough that an oocyte achieves after resuming meiosis, at Post LH surge and before each ovulation and is an indicator of nuclear maturation. With GVBD, first polar body, which comprises half number of chromosomes, protrudes from the cell, and the cell cycle is once more stopped at the metaphase-II. Oocyte maturation is the return of the first meiotic division, progression to M-II, expansion of the cumulus, and ensuing cytoplasmic modifications necessary for the oocyte to be capable of appropriate fertilization (Smith, 2001) [48]. The newly created embryo's first mitotic cell division is triggered by ooplasm, which also encourages the formation of the male pronucleus and rewires the nucleus of the fertilizing spermatozoon. Nuclear, cytoplasmic, and membrane components are all necessary for complete oocyte maturation (Eppig, 1996) [12].

It is simple to recognize that maturation of nuclear requires the progression and continuation of meiosis up until the metaphase-II. Chromosomal segregation is primarily involved in nuclear maturation, whereas organelle reconfiguration and storage of mRNAs, proteins, and transcription factors that are involved in the overall maturation, fertilization, and embryogenesis are involved in cytoplasmic maturation.

All oocytes are ineligible to undergo fertilization to begin early embryo development at the meiotic competence stage. In the course of follicular expansion, acquisitions appear to be progressive. Throughout folliculogenesis, the proportion of capable oocytes rises, but only a small number of oocytes complete the process by becoming fully competent, as the majority of follicles are lost due to physiological atresia that takes place throughout the growth phase. According to Mermillod *et al*. (2008) [34], the progression of the proportion of competent oocytes in the population of developing follicles is explained by the accumulation of factors (messengers, proteins) essential to the success of early developmental steps, the selection of healthy oocyte-containing follicles in increasingly selective hormonal environments, and challenging interfollicular regulation.

Physiological factors

a. Effect of follicle size and stage of follicular wave

The amount of blastocyst cells was unaffected by follicle size. The rates of meiosis resumption, the pattern of protein neosynthesis, and the transcriptome profile were unaffected by follicle size. Small follicle-derived blastocysts experienced a delayed cavitation and growth. Although no differences could be seen directly at the oocyte level, higher developmental competence for oocytes from follicles 6 mm versus 4 mm was shown in lab experiments (Lequarre *et al*., 2005) [26].

Pavlok *et al*. (1992) [39] observed that bovine oocytes taken from follicles with a diameter of less than 2 mm did not progress to the blastocyst stage. Oocytes recovered from follicles 2-4 mm and 4-8 mm in diameter have been demonstrated to have equivalent developmental capabilities. However, no change in oocyte developmental potential in cattle follicles with diameters of 2 mm and larger (Pavlok *et al*., 1993; Tan & Lu, 1990) [40, 50].

In both the growth phase and the dominant phase, the mean number of all counted follicles and the total number of usable oocytes collected per donor were comparable, but the mean number of embryos per donor and the rate at which oocytes developed into blastocysts were higher in the growth phase than in the dominant phase (p<0.01). In the dominant phase, which was less efficient for oocyte collection, both the quantity of oocytes from medium follicles and the developmental competency of oocytes from small follicles decreased (Machatkova *et al*., 2004) [28]. Oocyte developmental competence is influenced by the follicular wave, stage at which OPU is performed. Ability of an egg to develop into a blastocyst on days 2 and 5 of the follicular wave was high (27 percent and 29 percent, respectively), but low on day 8 (15 percent). This suggests that at a somewhat late stage of dominance, the dominant follicle lowers the developmental competence of oocytes from inferior follicles. The dominant follicle formed 3 to 5 days following the start of a new wave and reached its greatest size on day 5 (Hendriksen *et al*., 2004) [16].

The competence of oocytes from subordinate follicles is negatively impacted by the dominant follicle (Hagemann *et al*., 1999) [15]. Dominant follicle puncture was used to start the follicular wave in heifers at various points of the oestrous cycle. In the absence of a dominant follicle the proportion of oocytes that matured into blastocysts was higher (day 2 and 10: 44.8 percent blastocysts), than in the presence of a dominant follicle (day 7 and 15: 36.0 percent blastocysts). PG induced oestrus resulted in considerably low blastocyst rate in oocytes collected on day 1 than on days 2 and 3 (12.8 versus. 27.8 and 27.5 percent, respectively) (Machatkova *et al*., 2000) [27].

The prematuration theory, which holds that oocytes acquire developmental competence during the prematuration stage, provides a plausible explanation for the findings. Despite the fact that prematurity can occur during follicular atresia, it usually does so at the conclusion of preovulatory development, just before the LH surge (Sirard *et al*., 1999) [47].

b. Effect of oocyte donor age

Oocyte competence is affected by the age of the female donor. Compared to oocytes recovered from sexually mature females, oocytes recovered from prepubertal females have lower developmental potential (Ledda *et al*., 2001) [25]. In cattle (Revel *et al*., 1995) [44], pigs (Grupen *et al*., 2003) [14], and sheep (O'Brien *et al*., 1997) [38], the rate of blastocyst development is lowered in prepubertal oocytes. Prepubertal female porcine oocytes that reach the blastocyst stage result in embryos with less trophectoderm and total cells (Grupen *et al*., 2003) [14] and lower birth rates in sheep (Quirke & Hanrahan, 1977) [43] than mature female oocytes. There is evidence of early cleavage failure and an inability to resolve the maternal-embryonic transition (Armstrong, 2001) [2].

Visual and subjective ovarian morphology in cattle up to 17 years of age reveals the impact of age on ovarian morphology indicate a decrease in the number of follicles but no differences in oocyte quality (Kątska & Smorag, 1984) [22]. Studies demonstrated that embryonic development in cows older than 12 years is declining (Dias *et al*., 2014) [8]. Malhi *et al*. (2005) [30] found that in aged cows, the number of follicles recruited per follicular wave was lower, circulating estrogen concentrations were higher during the follicular phase, and CL diameter and progesterone concentrations were lower than in young cows. In older cows with two follicular wave cycles, the ovulatory follicle width gets shrunk (Malhi *et al*., 2005) [30]. Due to a reduced number of follicles recruited and their ability to expand successively into larger categories, aged cows experienced fewer ovulations than young cows, in a study utilizing a super ovulatory animal model (Malhi *et al*., 2006, 2008) [32, 31]. Oocyte competence was impaired in older cows, resulting in fewer embryos and a larger proportion of unfertilized oocytes than in younger cows (38 versus 71 percent) (Malhi, 2007) [29]. During the processing of *in vitro* embryos, oocytes collected from elderly cows showed reduced growth, with less capacity to mature and fertilize successfully (Iwata *et al*., 2011) [18]. Age of the cow and cleavage status are negatively correlated. Results clearly demonstrate that aged cattle have lower oocyte competence (Takeo *et al*., 2013) [49]. In donors aged 5 to 8 months, the total number of follicles was considerably greater (p<0.05), and in donors aged 9 months, the total number of follicles tended to be higher (p = 0.053). Fewer embryos reached the Morula and blastocyst stages when oocytes from donors aged 5 to 10 months were used. Younger animals can produce more oocytes, but their lower developmental competence cancels out this benefit (Landry *et al*., 2016) [24].

c. Developmental competence of OPU derived versus abattoir derived oocytes

Technique of oocyte retrieval has an impact on their developmental capacity. Increased developmental capability of oocytes produced from OPU versus slaughterhouse matured has been hypothesized to be owing to the little contact to environmental strain (Neglia *et al*., 2003) [36]. The time between peritoneal cavity ablation and laboratory processing is greater for oocytes obtained from slaughterhouses, and they are more prone to cellular damage caused by autolytic processes, particularly when they are kept in excised ovaries for an extended period of time. Oocytes acquired by OPU mature faster in a maturation medium than ovaries collected from abattoirs. Follicular population decreases to zero by aspirating all visible ovarian follicles during OPU, a fresh follicular wave starts, and in succeeding follicular aspiration 4 days later does not allow dominance to be established, results in enhanced oocyte competence and greater embryo production (Boni *et al*., 1994) [4]. OPU-derived blastocysts have considerably larger amount of inner cell mass (ICM) cells than the slaughterhouse-derived oocytes (Karadjole *et al*., 2010) [21]. Number of inner and outer cell mass of blastocyst is a critical element in optimal development.

d. Effect of OPU frequency on developmental competence

There was no difference in the typical COCs/follicles retrieved, however the recovery rate of the oocyte was higher after a twice-weekly aspiration session than after a once-weekly one (48.24 versus 71.08%). The frequency of oocyte collection, however, affects the developmental competence of *in vitro* oocytes. OPU sessions result in higher efficiency with twice a week rather than once a week (23 versus 11 percent) (Ding *et al*., 2008). Oocytes collected at a distance of 3-4 days twice a week rather than once a week, resulted in higher number of good-quality oocytes, those are capable of maturing to the blastocyst level (Merton *et al*., 2003) [35]. Oocytes collected twice a week lead to more homogeneous oocyte population and better quality (Merton *et al*., 2003) [35]. OPU conducted once a week instead of every 3–4 days resulted in lower blastocyst rates. Dominant follicle is unlikely to exist due to increased OPU frequency (Petyim *et al*., 2003) [41]. According to Salamone *et al*. (1999) [45], In comparison to the first wave, COC competency increased. Compared to COCs on days 1-2 or 3-4 of the follicular wave, COCs on days 5 and 6 of the follicular wave had a higher incidence of expanded cumulus. The best recovery and oocyte quality of a donor cow could be achieved with twice-weekly OPU sessions and a three-month rest period, respectively (Kang *et al*., 2019) [20].

e. Role of cumulus cells

Oocytes containing above three layers of cumulus cells exhibit maximum maturation rate irrespective of their retrieval method. The maturation of bovine oocytes is significantly influenced by COC morphology (Kakkassery *et al*., 2010) [19]. COCs with different cumulus cell layers (up to five layers), that are compact or slightly enlarged, with or without dark regions in the oocyte and cumulus, are the best selection points (Aguila *et al*., 2020) [1]. Lack of COC during IVM impairs the oocyte's lipid metabolism and causes less-than-ideal cytoplasmic maturation. By controlling local fatty acid synthesis and lipolysis to supply energy for maturation, COC direct the oocyte's use of nutritional store (Auclair *et al*., 2013) [3]. Cumulus cell presence and glutathione (GSH-Antioxidant) content are closely connected (Yamauchi & Nagai, 1999) [51]. The stimulatory effect of cysteine and cysteamine on GSH synthesis in Cumulus Oocyte Complexes (COC's) was influenced by cumulus cells.

Oocyte maturation, fertilization and *in vitro* developmental stages were significantly slower when cumulus cells were eliminated before maturation. Cumulus cells removed before IVF or 7 hours after IVF decreased fertilization, cleavage development, morula and blastocyst formation. Cumulus cell elimination at 20 h after IVF produced development that was comparable to controls at all stages. On or before 7 hours after IVF, the COC's appear to be advantageous, but not necessary, by 20 hours following IVF (Zhang *et al*., 1995) [53].

Laboratory factors

Apart from the physiological factors, there are laboratory factors like, effect of media (Tissue culture media-199, synthetic oviduct fluid-SOF), serum (foetal calf serum-FCS), gas atmosphere (5% O2, 90% N2 and 6% CO2) and duration of maturation influence the *in vitro* maturation of bovine oocyte. Addition of FSH, LH, estrogens and antioxidants in the maturation media has been linked to the ability to be fertilized and grow normally *in vitro* and it may not even represent the true follicular environment during *in vivo* maturation (Younis *et al*., 1989) [52].

Addition of foetal calf serum (FCS), hormones, and other beneficial components to the maturation medium significantly increases mature oocyte quality (Brackett & Zuelke, 1993) [6]. Among the several serum components, glucose has a critical role in maturation. In the absence of glucose in the serum, ovum resumes meiosis but unable to reach M-II (Kimura *et al*., 2008) [23]. Biologically active substances, such as FSH, and other growth factors are added to *in vitro* maturation techniques to enhance oocyte and embryo quality (Brackett, 2001) [5].

Period of IVM plays a key role in affecting the generation of mammalian embryos *in vitro* (Marston & Chang, 1964) [33], due to improper maturation timing leads to aberrant chromatin (Dominko & First, 1997) [10], oocyte ageing (Hunter & Greve, 1997) [17] and diminished growth. Sperm can reach oocytes before they are fully matured (Chian *et al*., 1992) [7], and subsequent development is often limited. The finest time for IVF is at conclusion of meiosis, which can take anywhere between 18 and 24 hours in cattle (Sirard *et al*., 1989; Neglia *et al*., 2001) [46, 37] and 36 to 48 hours in pigs (Prather & Day, 1998) [42] depending on the species.

Conclusion

Although achieving established parameters is a laudable objective, it is evident that blastocysts produced by IVM are of subpar quality, as it is almost impossible to control the physiological factors and mimic *in vivo* maturation. However, laboratory factors *viz*, maturation media and supplements, duration of maturation, gaseous condition (incubator setup) can be controlled and *in vitro* maturation can be improved.

References

1. Aguila L, Treulen F, Therrien J, Felmer R, Valdivia M, Smith LC. Oocyte selection for *in vitro* embryo production in bovine species: noninvasive approaches for new challenges of oocyte competence. Animals. 2020;10(12):2196.

2. Armstrong DT. Effects of maternal age on oocyte developmental competence. Theriogenology. 2001;55(6):1303-1322.
3. Auclair S, Uzbekov R, Elis S, Sanchez L, Kireev I, Lardic L, *et al*. Absence of cumulus cells during *in vitro* maturation affects lipid metabolism in bovine oocytes. American Journal of Physiology-Endocrinology and Metabolism. 2013;304(6):E599-E613.
4. Boni R, Di Palo R, Barbieri V, Zicarelli L. Ovum pick-up in deep anestrus buffaloes. Proc. IV World Buffalo Congress. 1994;(3):480-482.
5. Brackett BG. Advances in animal *in vitro* fertilization. Assisted Fertilization and Nuclear Transfer in Mammals; c2001. p. 21-51.
6. Brackett BG, Zuelke KA. Analysis of factors involved in the *in vitro* production of bovine embryos. Theriogenology. 1993;39(1):43-64.
7. Chian RC, Niwa K, Nakahara H. Effect of sperm penetration *in vitro* on completion of first meiosis by bovine oocytes arrested at various stages in culture. Reproduction. 1992;96(1):73-78.
8. Dias FCF, Khan MIR, Adams GP, Sirard MA, Singh J. Granulosa cell function and oocyte competence: Super-follicles, super-moms and super-stimulation in cattle. Animal Reproduction Science, 2014;149(1-2):80-89.
9. Ding LJ, Tian HB, Wang JJ, Chen J, Sha HY, Chen JQ, Cheng GX. Different intervals of ovum pick-up affect the competence of oocytes to support the preimplantation development of cloned bovine embryos. Molecular Reproduction and Development: Incorporating Gamete Research. 2008;75(12):1710-1715.
10. Dominko T, First NL. Timing of meiotic progression in bovine oocytes and its effect on early embryo development. Molecular Reproduction and Development: Incorporating Gamete Research. 1997;47(4):456-467.
11. Duszewska AM, Reklewski Z. Reproduction of cattle using the OPU-IVP-ET. Prace i Materiały Zootechniczne. 2000;57:111-120.
12. Eppig JJ. Coordination of nuclear and cytoplasmic oocyte maturation in eutherian mammals. Reproduction, Fertility and Development. 1996;8(4):485-489.
13. Gasparrini B, De Rosa A, Attanasio L, Boccia L, Di Palo R, Campanile G, *et al*. Influence of the duration of *in vitro* maturation and gamete co-incubation on the efficiency of *in vitro* embryo development in Italian Mediterranean buffalo (*Bubalus bubalis*). Animal Reproduction Science. 2008;105(3-4):354-364.
14. Grupen CG, McIlfatrick SM, Ashman RJ, Boquest AC, Armstrong DT, Nottle MB. Relationship between donor animal age, follicular fluid steroid content and oocyte developmental competence in the pig. Reproduction, Fertility and Development. 2003;15(2):81-87.
15. Hagemann LJ, Beaumont SE, Berg M, Donnison MJ, Ledgard A, Peterson AJ, *et al*. Development during single IVP of bovine oocytes from dissected follicles: interactive effects of estrous cycle stage, follicle size and atresia. Molecular Reproduction and Development: Incorporating Gamete Research. 1999;53(4):451-458.
16. Hendriksen PJM, Steenweg WNM, Harkema JC, Merton JS, Bevers MM, Vos PLAM, *et al*. Effect of different stages of the follicular wave on *in vitro* developmental competence of bovine oocytes. Theriogenology. 2004;61(5):909-920.
17. Hunter RHF, Greve T. Could artificial insemination of cattle be more fruitful? Penalties associated with ageing eggs. Reproduction in Domestic Animals. 1997;32(3);137-141.
18. Iwata H, Goto H, Tanaka H, Sakaguchi Y, Kimura K, Kuwayama T, *et al*. Effect of maternal age on mitochondrial DNA copy number, ATP content and IVF outcome of bovine oocytes. Reproduction, Fertility and Development. 2011;23(3):424-432.
19. Kakkassery MP, Vijayakumaran V, Sreekumaran T. Effect of cumulus oocyte complex morphology on *in vitro* maturation of bovine oocytes. Journal of Veterinary and Animal Sciences. 2010;41:12-17.
20. Kang SS, Kim UH, Lee SD, Lee MS, Han MH, Cho SR. Recovery Efficiency of Cumulus Oocyte Complexes (COCs) according to Collection Frequency for Ovum Pick-up (OPU) Method in Hanwoo Cow. Journal of Animal Reproduction and Biotechnology. 2019;34(4):300-304.
21. Karadjole M, Getz I, Samardžija M, Maćešić N, Matković M, Makek Z, *et al*. The developmental competence of bovine immature oocytes and quality of embryos derived from slaughterhouse ovaries or live donors by ovum pick up. Veterinarski Arhiv. 2010;80(4):445-454.
22. Kątska L, Smorąg Z. Number and quality of oocytes in relation to age of cattle. Animal Reproduction Science. 1984;7(5):451-460.
23. Kimura K, Iwata H, Thompson JG. The effect of glucosamine concentration on the development and sex ratio of bovine embryos. Animal Reproduction Science. 2008;103(3-4):228-238.
24. Landry DA, Bellefleur AM, Labrecque R, Grand FX, Vigneault C, Blondin P, *et al*. Effect of cow age on the *in vitro* developmental competence of oocytes obtained after FSH stimulation and coasting treatments. Theriogenology. 2016;86(5):1240-1246.
25. Ledda S, Bogliolo L, Leoni G, Naitana S. Cell coupling and maturation-promoting factor activity in *in vitro*-matured prepubertal and adult sheep oocytes. Biology of Reproduction. 2001;65(1):247-252.
26. Lequarre AS, Vigneron C, Ribaucour F, Holm P, Donnay I, Dalbies-Tran R, *et al*. Influence of antral follicle size on oocyte characteristics and embryo development in the bovine. Theriogenology. 2005;63(3):841-859.
27. Machatkova M, Jokesova E, Horky F, Krepelova A. Utilization of the growth phase of the first follicular wave for bovine oocyte collection improves blastocyst production. Theriogenology. 2000;54(4):543-550.
28. Machatkova M, Krausova K, Jokesova E, Tomanek M. Developmental competence of bovine oocytes: effects of follicle size and the phase of follicular wave on *in vitro* embryo production. Theriogenology. 2004;61(2-3):329-335.
29. Malhi PS. A bovine model to study reproductive aging (Doctoral dissertation); c2007.
30. Malhi PS, Adams GP, Singh J. Bovine model for the study of reproductive aging in women: follicular, luteal, and endocrine characteristics. Biology of Reproduction. 2005;73(1):45-53.
31. Malhi PS, Adams GP, Mapletoft RJ, Singh J. Superovulatory response in a bovine model of reproductive aging. Animal Reproduction science. 2008;109(1-4):100-109.
32. Malhi PS, Adams GP, Pierson RA, Singh J. Bovine

model of reproductive aging: response to ovarian synchronization and superstimulation. Theriogenology. 2006;66(5):1257-1266.
33. Marston JH, Chang MC. The fertilizable life of ova and their morphology following delayed insemination in mature and immature mice. Journal of Experimental Zoology. 1964;155(2):237-251.
34. Mermillod P, Dalbiès-Tran R, Uzbekova S, Thelie A, Traverso JM, Perreau C, *et al*. Factors affecting oocyte quality: who is driving the follicle? Reproduction in Domestic Animals. 2008;43:393-400.
35. Merton JS, De Roos APW, Mullaart E, De Ruigh L, Kaal L, Vos PLAM, *et al*. Factors affecting oocyte quality and quantity in commercial application of embryo technologies in the cattle breeding industry. Theriogenology. 2003;59(2):651-674.
36. Neglia G, Gasparrini B, di Brienza VC, Di Palo R, Campanile G, Presicce GA, *et al*. Bovine and buffalo *in vitro* embryo production using oocytes derived from abattoir ovaries or collected by transvaginal follicle aspiration. Theriogenology. 2003;59(5-6):1123-1130.
37. Neglia G, Marino M, Di Palo R, Wilding M, Caracciolo di Brienza V, Dale B, *et al*. A comparison of *in vitro* maturation in buffalo (*Bubalus bubalis*) and bovine oocytes using confocal microscopy. Theriogenology. 2001;55:488.
38. O'Brien JK, Catt SL, Ireland KA, Maxwell WMC, Evans G. *In vitro* and *in vivo* developmental capacity of oocytes from prepubertal and adult sheep. Theriogenology. 1997;47(7):1433-1443.
39. Pavlok A, Kopečný V, Lucas Hahn A, Niemann H. Transcriptional activity and nuclear ultrastructure of 8 cell bovine embryos developed by *in vitro* maturation and fertilization of oocytes from different growth categories of antral follicles. Molecular Reproduction and Development. 1993;35(3):233-243.
40. Pavlok A, Lucas-Hahn A, Niemann H. Fertilization and developmental competence of bovine oocytes derived from different categories of antral follicles. Molecular Reproduction and Development. 1993;31(1):63-67.
41. Petyim S, Båge R, Hallap T, Bergqvist AS, Rodríguez-Martínez H, Larsson B. Two different schemes of twice-weekly ovum pick-up in dairy heifers: effect on oocyte recovery and ovarian function. Theriogenology. 2003;60(1):175-188.
42. Prather RS, Day BN. Practical considerations for the *in vitro* production of pig embryos. Theriogenology. 1998;49(1):23-32.
43. Quirke JF, Hanrahan JP. Comparison of the survival in the uteri of adult ewes of cleaved ova from adult ewes and ewe lambs. Reproduction. 1977;51(2):487-489.
44. Revel F, Mermillod P, Peynot N, Renard JP, Heyman Y. Low developmental capacity of *in vitro* matured and fertilized oocytes from calves compared with that of cows. Reproduction. 1995;103(1):115-120.
45. Salamone DF, Adams GP, Mapletoft RJ. Changes in the cumulus-oocyte complex of subordinate follicles relative to follicular wave status in cattle. Theriogenology. 1999;52(4):549-561.
46. Sirard MA, Florman HM, Leibfried-Rutledge ML, Barnes FL, Sims ML, First NL. Timing of nuclear progression and protein synthesis necessary for meiotic maturation of bovne oocytes. Biology of Reproduction. 1989;40(6):1257-1263.
47. Sirard MA, Picard L, Dery M, Coenen K, Blondin P. The time interval between FSH administration and ovarian aspiration influences the development of cattle oocytes. Theriogenology. 1999;51(4):699-708.
48. Smith GD. *In vitro* maturation of oocytes. Current Women's Health Reports. 2001;1(2):143-151.
49. Takeo S, Goto H, Kuwayama T, Monji Y, Iwata H. Effect of maternal age on the ratio of cleavage and mitochondrial DNA copy number in early developmental stage bovine embryos. Journal of Reproduction and Development. 2013;59(2):174-179.
50. Tan SJ, Lu H Effects of different oestrous stages of ovaries and sizes of follicles on generation of bovine embryos *in vitro*. Theriogenology. 1990;33(1):335.
51. Yamauchi N, Nagai T. Male pronuclear formation in denuded porcine oocytes after *in vitro* maturation in the presence of cysteamine. Biology of Reproduction. 1999;61(3):828-833.
52. Younis AI, Brackett BG, Fayrer-Hosken RA. Influence of serum and hormones on bovine oocyte maturation and fertilization *in vitro*. Gamete Research. 1989;23(2):189-201.
53. Zhang LI, Jiang S, Wozniak PJ, Yang X, Godke RA. Cumulus cell function during bovine oocyte maturation, fertilization, and embryo development *in vitro*. Molecular Reproduction and Development. 1995;40(3):338-344.