ORIGINAL ARTICLE Open Access

ISSN 2278-1404

International Journal of Fundamental & Applied Sciences

The promising and potential role of IVF in cattle and beef industry

Debasmita Debnath, Ipsita Sinha, Kumari Khushboo, Vedamurthy AB, Mamatha J*
P.G. Department of Biotechnology, The Oxford College of Science,
Bangalore 560102, Karnataka, India.

Abstract

Background and Objective : In this work, dissociation of nano drug 5-Fluorouracil derivatives was studied theoretically. ***Methodology*** : For this purpose, Gibbs free energy values for neutral and deprotonated forms of 5-Fluorouracil were calculated at gas and aqueous phases by using density functional theory (DFT) method. Solvent effects are taken into account by means of polarizable continuum model (PCM). ***Result*** : It was shown that, theoretically calculated pK_a values are in good agreement with the existing experimental pK_a values, which are determined from capillary electrophoresis, potentiometric titration and UV–visible spectrophotometric measurements.

Keywords: Sperm, Surrogate mother, Embryo, Oocytes, *IVF*.

1. Introduction

In vitro fertilization basically involves fertilization of oocytes of a female animal with a male animal under laboratory condition. *IVF* is reasonably successful as it results in 70%-80% of the fertilized egg[1]. Through *IVF* technology a large number of off-springs can be produced from a single animal, thus a female animal, which normally produce 4-5 off-springs in her life time, can produce as many as 20-50 off-springs by employing *IVF*. The production of cattle from *In- vitro* procedure became established commercially on a international basis during the 1990s. The pregnancy rates resulting from transfer of *In-vitro* derived embryos have average 50% at 60 days of gestation period, slightly lower than the pregnancy rate from the *In-vivo* embryos. It can be used to improve pregnancy rate in herds with low fertility or certain reproductive breakdown, such as ovulation and fertilization failure or reproductive tract blockage. The problem with *IVF* includes heavier birth rate, extended gestation period, higher rate of abortion etc. The different steps of *In-vitro* production of cattle embryos up to the blastocyst stage in semidefined condition includes oocytes[2] maturation, *IVF* and *In-vitro* development. Sometime oocytes are associated with maturational and developmental abnormalities[3]. *IVF* can be valuable instrument in the ability to assist genetic selection, Strategies and breeding plans for cattle production system. Rate of selection for quantitative traits can be increased by exploitation of in vitro embryo technologies to improve the accuracy and intensity of selection and to reduce the generation interval[1] .Crossbreeding of cattle has received renewed interest for dairy production systems[4]. *In- vitro* produced Cattle embryos play a central role in dairy and beef production systems. The historical decline in pregnancy rates of dairy cattle[5,6,7] makes the search for solutions to infertility compelling. Research is going on to improve the technical aspects of *In-vitro* fertilization. Therfore, In this study, dissociation of nano drug 5-Fluorouracil derivatives was studied by the authors.

2. Methodology

2.1. Media and Reagents:

All the media used for this method was provided by Sigma Aldrich chemical (St.Louis,MO,USA). Normal saline solution (1000ml) was used for storing the ovaries after they were collected from the slaughter house. Aspiration media (100 ml) was used during oocytes collection from ovaries. Washing media (40ml) used for removing the excess cumulous cell. Maturation media (10ml) for maturation of the oocytes, *IVF* media (solution A – 500ml and solution B- 200ml) , Working BO media (50ml) and BO (Brackett and Oliphant) medium for capacitation and Fertilization, EDM (embryo development media) (10 ml) Primary medium for embryo development, Replacement media for better nourishment of newly formed embryo.

2.2. Collection of Ovaries

Cattle ovaries were collected from Kolkata, India slaughterhouse and were transported within 3-4 hour to the lab in the normal saline at 30-35˚C. Ovaries were then trimmed very carefully by using sterile surgical scissors (Figure I)

Corresponding author
Full Address :
The Oxford College of Science,
Bangalore 560102, Karnataka, India
Phone no. +91-9738109917
E-mail: *mamathaj.raj@gmail.com*

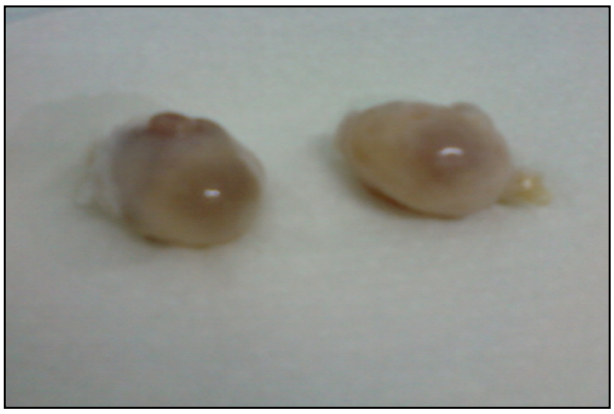

Figure I: Ovaries collected From Cattle

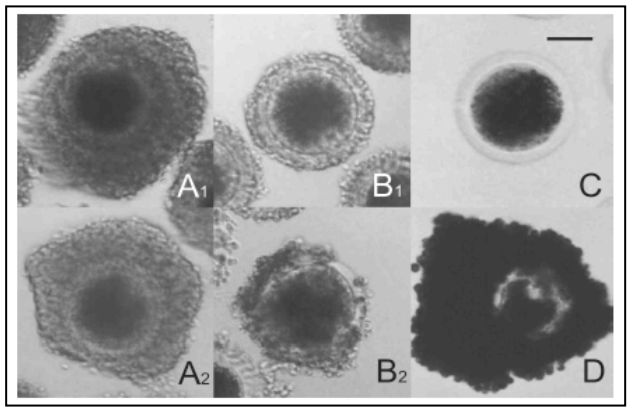

Figure II: Grading of Oocytes

Table I: Grading of Oocytes

Total number of Ovaries	Total number of Oocytes collected	Total number of Oocytes collected in different grade			
		A	B	C	D
37	46	6	8	12	20

2.3 Retrieval of Oocytes

Folliculer oocytes were collected from ovaries adopting the aspiration method. In this method oocytes were collected from the visible surface follicles of ovaries with a 19 gauge hypodermic needle attached to a 5 ml disposable plastic syringe. Some amount of aspiration media was also taken in the syringe before aspiration.

2.4 Searching of Oocytes

The oocytes in the aspiration media were searched and collected from the aspiration media under the stereo-zoom microscope. The oocytes were graded on the basis of the presence of cumulus cells.

2.5 Grading of Oocytes

After collection, the oocytes were graded on the basis of presence of cumulus cells surrounding the oocytes, grade A- oocytes having 4-5 layers of cumulus cells, grade B- oocytes having 2-3 layers of cumulus cells, grade C- oocytes having the single layer or incompletely covered with cumulus cells and grade D or nude oocytes with no cumulus cells. After grading, the oocytes were washed thoroughly (5-6 times) in the washing medium (Figure II), (Table I).

2.6 Maturation of oocytes

Growth and maturation of both male and female gametes culminates in production of fertilization-competent eggs .Investigators demonstrate that sperm-egg recognition depends on the cleavage status of ZP2[8].The oocytes were then placed in the maturation media, the maturation media was filled with a disposable microfilter having a pore size of 0.22µm.The maturation media was also supplimented with cumulus cells. The oocytes were placed in the maturation media droplet (100µl) covered with sterile prewarmed paraffin oil. It was then incubated for 24 hour at 38˚C for their *in- vitro* maturation.

2.7 Sperm Preparation

Semen straws were taken out from liquid nitrogen, cryocan and proper thawing was done by putting straws in the water at room temperature. The physiological changes that confer on the sperm the ability to fertilize are collectively called "capacitation." Capacitation was first described and defined independently by Chang and Austin[9,10]. Semen were then placed in the capacitation media and repeated centrifugation was done to collect the most motile sperms.

2.8 Oocytes preparation after IVM

After 24 hours the *in vitro* matured Oocytes were taken out from the Co_2 incubator. Matured Oocytes with expanded cumulus cells were found. Then repeated pipetting was done to remove the cumulus cells.

2.9 *In vitro* fertilization (*IVF*)

First of all sperm capacitation was an essential Prerequisite for fertilization of ova *in vitro*. The, oocytes were washed repeatedly in the washing media and were placed in the fertilization media and inseminated with capacitated sperm and incubated for 14 hours at 38.5˚C.

2.10 Embryo development

Several studies have indicated that the frequency of multiple births in cattle can be increased by gonadotropin hormonal therapy. After fertilization, Oocytes were placed in the embryo development media (EDM) for 48 hours at 38.5˚C.The embryos developed were then transfer to the replacement media supplemented with serum, which would provide further nutrition and enhance the rate of cell-division.

2.11 Vitrification and Cryopreservation

Vitrification procedures were initially described by Zhou *et al* [11]. The embryos were then loaded in to open pulled straws (OPS)[12,15]each containing DPBS. Approximately 10 oocytes were picked up by the narrow end of OPS within 25 s and the OPS were immediately plunged into LN2. The straws were sealed and then pre-cooled by keeping them into liquid nitrogen.

3. Results and Discussion

Total 46 immature oocytes were isolated from 37 ovaries which were collected from slaughter house. Out of that 19 Oocytes were matured and fertilized *In- vitro*. Total 7 embryos were developed after culturing in the culture media (Figure III), (Table II). *In vitro* development proceeded to the 8-cell stage. Vigorous progressive sperm motility and acrosome integrity were important features of good sperm samples capable of penetrating zona-pellucida of oocytes[14]. If Compared with *in vivo* derived embryos, cattle embryos derived from IVF, in general, appear different by light and electron microscopy, differ in number of cells, size, developmental rate, temperature sensitivity, freezability, viability, and pregnancy rates after transfer . The latest statistics reported by the International Embryo Transfer Society noted over 30,000 IVP embryos were transferred into recipient cows in 1997 [16].

Table II: Frequency of embryo development

Number of Ovaries	Total number of oocytes collected	Total number of oocytes used for *in-vitro* maturation	Total number of oocytes matured	Total number of embryos developed
37	46	46	19 (41%)	7 (36%)

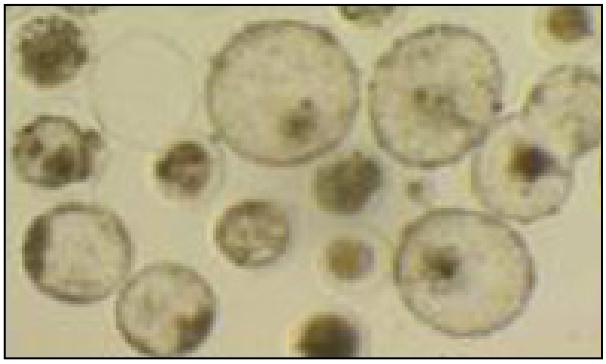

Figure III: Different Stages of Cattle Embryo

The fertilization rate of vitrified oocytes with frozen-thawed spermatozoa was lower than that of fresh oocytes indicating that OPS vitrification resulted in decreased fertilizing ability in oocytes as well as could induce premature release of cortical granule contents[17,18]. The *In-vitro* produced embryos play a major role in genetic selection scheme based on allelic variance of specific gene. *In-vitro* produced embryos can be screened for inheritance of specific alleles, so that genetic selection can be made before pregnancy is established. It was revealed that it is not necessary to superovulate the cattle oocytes and on the other hand a cattle can be aspirated every 20 days. *IVF* promises to provide enormous benefit to biotechnology, biomedicine, farm animal breeding and research. Recent advancement in IVF and its related fields with the tools of molecular biology opens a new horizon in reproductive biotechnology[13]. It also allows improving designing and testing of future therapies for managing reproduction, with application for agricultural, transgenesis and endangered species, providing models for human disease. Progress in IVF is evidenced by the ability to achieve gamete union and pregnancies following embryo transfer in common laboratory and domestic animals. Additionally, defined conditions are available for support of oocyte maturation, sperm capacitation, IVF, and embryo culture for several species. These and other advances in complementing technologies provide great impetus for acceleration of additional refinements in IVF to afford better ways to enhance reproductive efficiency and to understand physiological events at the molecular level. Greater prominence can be anticipated for IVF in research and practical applications.

Acknowledgement

We are thankful to Dr. S.K. Das, Senior Scientist (Animal Biotechnology), National Dairy Research Institute (NDRI), Kalyani, WB, India for the valuable guidance. We are also grateful to The Oxford College of Science, Bangalore for the Support and Cooperation.

Conflict of Interest :

Authors don't have any conflict of interest.

References:

1. Hansen PJ, & Block J, Towards an embryocentric world: the current and potential uses of embryo technologies in dairy production. *Reprod Fertil Dev,* **16** (2004) 1–14.
2. Hubner K, Fuhrmann G, Christenson LK, Kehler J, Reinbold R, de la Fuente R, *et al.* Derivation of oocytes from mouse embryonic stem cells. *Science,* **300** (2003) 1251–1256.
3. Herbert M., & Gillespie J.I., and Murdoch A.P. development of calcium signalling mechanisms during maturation of human oocytes. *Molecular Human Reproduction* **3** (1997) 965-973
4. McAllister AJ. Is crossbreeding the answer to questions of dairy breed utilization? *J Dairy Sci,* **85** (2002) 2352–2357.
5. Royal MD, Darwash AO, Flint APF. Webb R,Wooliams JA, & Lamming GE, Declining fertility in dairy cattle: changes in traditional and endocrine parameters of fertility. *Anim Sci,* **70** (2000) 487–501.
6. Lucy MC, Reproductive loss in high-producing dairy cattle: where will it end? *J Dairy Sci,* **84** (2001) 1277–1293.
7. Al-Katanani YM, Drost M, Monson RL, Rutledge JJ, Krininger III CE, Block J, *et al*., Pregnancy rates following timed embryo transfer with fresh or vitrified in vitro produced embryos in lactating dairy cows under heat stress conditions. *Theriogenology,* **58** (2002) 171–182.
8. Gahlay G, Gauthier L, Baibakov B, Epifano O, & Dean J, *Gamete recognition in cattle depends on the cleavage status of an egg's zona pellucida protein*, *Science*, **329**(2010) 216–219.
9. Chang MC, Development of fertilizing capacity of rabbit spermatozoa in the uterus. *Nature,* **175** (1955)1036–1037.
10. Austin CR, Observations on the penetration of the sperm into the mammalian egg. *Aust J Sci Res* **4**(1951)581–596.
11. Zhou GB, Hou YP, Jin F, *et al*., Vitrification of mouse embryos at various stages by open-pulled straw(OPS) method. *Anim Biotechnol*, **16** (2005) 153–163.
12. Vajta G, Booth PJ, Holm P, Greve T, & Callesen H. Successful vitrification of early stage bovine in vitro produced embryos with open pulled straw (OPS) method. *Cryo Letters,* **18** (1997)191–195
13. Nandi1 S, Kumar VG, & Chauhan MS, In vitro production of bovine embryos: we need to stop or proceed-A review. *Agri Rev,* **27** (2006) 122 – 129.
14. Brackett BG, Bousquet D, Boice ML, Donawick WJ, Evans JF, & Dressel MA, Normal development following *in vitro* fertilization in the cow. *Biol Reprod,* **27** (1982) 147–158.
15. Zhi Qiang Fan, Yan Ping Wang, Chang Liang Yan, Lun Suo, & Shi En Zhu, Positive effect of partial zona pellucida digestion on in vitro fertilization of mouse oocytes with cryopreserved spermatozoa. *Laboratory Animals,* **43** (2009) 72–77
16. Thibier M, The 1997 embryo transfer statistics from around the world: a data retrieval committee report. *Embryo Transfer Newslett,* **16** (1998) 17–20.
17. Vincent C, Pickering SJ, & Johnson MH, The hardening effect of dimethyl sulphoxide on the mouse zona pellucida requires the presence of an oocyte and is associated with a reduction in the number of cortical granules present. *J Reprod Fertil*, **89** (1990) 253 –259.
18. Carroll J, Depypere H, & Matthews CD, Freeze-thaw-induced changes of the *zona pellucida* explains decreased rates of fertilization in frozen-thawed mouse oocytes. *J Reprod Fertil,* **90** (1990) 547–53.