ASSESSMENT OF NUCLEAR MATURATION AND OOCYTE VIABILITY IN NORMAL AND REPEAT BREEDER CROSSBRED COWS BY USING FLUORESCENT STAINING

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Received : 10.08.2017
Accepted : 25.08.2017

Abstract

The study was carried out in 12 normal and 12 repeat breeder crossbred cows. These cows were further divided into four groups of six animals in each group. The animals were subjected to transvaginal oocyte recovery (TVOR) for a period of twomonths without any treatment at once weekly and twice weekly interval. Culture grade oocytes retrieved were subjected to in vitro maturation for 24hat 38.5°C and 5 per cent CO_2. Nuclear maturation was assessed with Hoechst 33342 stain and oocyte viability was assessed with fluorescein diacetate (FDA) stain. Results revealed that nuclear maturation based on Hoechst 33342 as 80.00 and 76.19 per cent respectively for normal and repeat breeders. Assessment ofviability of oocytes based on fluorescein diacetate (FDA) stain revealed that viability of oocytes in normal breeders (83.33 per cent) was higher than repeat breeiders (75 per cent). The present study revealed that nuclear maturation rate and oocyte viability is less in repeat breeders when compared to normal breeders.

Key words: - *Transvaginal oocyte recovery, Oocyte viability nuclear maturation, Hoechst 33342, Fluorescein diacetate (FDA)*

Repeat breeding syndrome has been researched extensively and has resulted in a better understanding of many of the factors influencing or leading to the condition. A less explored aspect of repeat breeding syndrome

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is the quality of oocytes in such animals. The difficulty in obtaining oocytes from live animals isa major huddle for such studies. Hoechst 33342 is a nuclear dye, which was first used in mammalian cells by Arndt- Jovin andJovin (1977) and Lelande*et al*. (1981) in mammaliancells. It was also used to show the spatial organisation of chromatin in live mouse oocytes and zygotes (Zuccotti*et al.,* 1995) and to distinguish the developmental stage of oocytes and preimplantation embryos (Gall *et al*., 1996). Fluorescein-diacetateis a non-polar molecule which enters the cell, hydrolyzed by cell esterases and fluorescein is produced. This polar compound cannot leave the cell because it is unable to pass through the intact cell membrane, and therefore accumulates in cytoplasm of the cell. Damaged cells however show a distinct loss of fluorescein through the cell membrane.Since Hoechst 33342 and FDAcan be used for assessing the nuclear maturation of oocytes and oocyte viability, respectively, the present research work was carried out to assess the of oocyte quality in normal and repeat breeders by using fluorescent staining techniques.

Materials and Methods

The present study was conducted in 12 normal and 12 repeat breeder crossbred cows between first and sixth parity belonging to University Livestock Farm and Fodder Research Development Scheme, Mannuthy during the period from September 2015 to March 2017. All the repeat breeder animals selected for the study were with a history of failure of conception even after three or more consecutive inseminations and the normal breeding animals were without any history of breeding problems. Before starting the experiment subclinical endometritis was ruled out by conducting white side test (Pateria and Rawal 1990). Animals were divided into four experimental groups with six animals in each group, group I and II comprise of normal animals and group III and IV consist of repeat breeder animals, in which TVOR was performed at once weekly and twice weekly interval respectively per standard procedures (Sakhong*et al.,*2012). Oocytes retrieved were graded based on the number of layers of cumulus cells and character of ooplasm the oocytes were divided into four classes and grade A and B oocytes were considered as culture grade oocytes.

Culture grade oocytes were selected for *in vitro* maturation studies and washed four times with the washing medium containing TCM 199 supplemented with 10 per cent FoetalBovine Serum (FBS), 0.68 mM L-glutamine, 0.8mM sodium pyruvate and 50µg/mL gentamicin sulphate and then it transferred to maturation media containing TCM 199 enriched with 10 µg/mL LH, 5 µg/mL FSH, 1 µg/mL oestradiol-17β, 50µg/mL sodium pyruvate, 3.5 µg/mL L-glutamine, 50 µg/mL gentamicin, 3 mg/mL Bovine Serum Albumin (BSA), 10 per cent FBS and 20 µg/mL epidermal growth factor. Culture condition set for the study was for 24 h at 38.5^{o}C and 5 per cent CO2 in air with 95 per cent relative humidity.

Matured oocytes were stained with Hoechst 33342 stain,after denudation, they were incubated in bicarbonate buffered TCM 199 with 5µg/mL Hoechst 33342 stain for 15 minutes. After washing these oocytes were stained and viewed under fluorescent microscope (Leica-2000DM, Germany) with excitation filters of 340-380 nm and suppression filter of 425 nm (Smith,1993).

For assessing oocyte viability staining was carried out with FDA stain, denuded oocytes were incubated with 2.5µg FDA/mL of DPBS supplemented with 5mg/mL BSA for 2 minutes at 38.5^{0}C in a dark room. After staining oocytes were washed three times in PBS supplemented with 5mg/mL BSA and observed under fluorescent microscope (Leica-2000DM) with excitation filters of 490nm and suppression filters of 515nm. Oocytesoocytes expressing bright green fluorescence were considered to be alive (Katsa and Smorag, 1985 and Liang *et al*. 2012).

Results and discussion

A total of 41 matured oocytes were stained withHoechst 33342 stain for assessing nuclear maturation (Table1). Maturation rate of oocytes harvested from normal breeders subjected to once weekly and

twice weekly TVOR were 75.00 and 83.33 per cent,respectively. The corresponding values in repeat breeders were 77.78 and 75.00 per cent. Overall maturation rate of oocytes harvested from normal cows were higher than those of repeat breeders (80.00vs76.19 per cent). However no significant difference was observed between normal and repeat breeders.

Hoechst 33342 is a membrane permeant stain which can stain live cells by binding with adenine thymine rich regions of DNA in the minor groove and on binding to DNA fluorescence increases (Chazotte (2011). In Hoechst 33342 staining, nuclear material showed fluorescence. Germinal vesicle was viewed as a round uniform bright disc. Germinal vesicles break down (GVBD) is characterized by more condensed nuclear material with dissolution of nuclear membrane. Metaphase I appeared like a single set of chromosomes in equatorial plane. Metaphase II appeared as two sets of chromosomes moving away from each other one as polar body and other as oocyte nucleus arrested at Metaphase II. A much lower maturation rate was observed by Magnus (2005), who reported that staining of oocytes retrieved from slaughter ovaries with Hoechst 33342 revealed that maturation rate as 66.67%. The increased maturation rate of oocytes with Hoechst33342 stain in the present study may be because of the selection of only culture quality oocytes for studies and procurement of oocytes from live animals.

A total of 38 matured oocytes were stained with fluorescein diacetate (FDA) stain for assessing the oocyte viability (Table 1). In normal breeders, viability of oocytes harvested at once weekly and twice weeklyTVOR was 87.5 and 80 per cent,respectively. The corresponding values in repeat breeders were 77.78 and 72.73 per cent. Overall oocyte viability in normal breeders (83.33 per cent) was higher than repeat breeders (75 per cent). Mohr and Trounson (1980) mentioned that FDA being non-polar compound could readily pass into cell where it is hydrolyzed by esterase enzyme into polar substance and it accumulates intracellularly (Rotman and Papermaster, 1966) which yields fluorescein. Hence FDA staining can be used to assess both esterase enzyme activity and membrane integrity. Overall viability rate in normal breeders (83.33) were higher than repeat breeders (75 per cent).Almost similar results were obtained by Magnus *et al*., (2005) who reported that bovine oocytes retrieved from abattoir derived ovaries by aspirationrevealed 80 per cent viability.

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Table 1: Nuclear maturation rate and oocyte viability of normal and repeat breeder crossbred cows subjected to different TVOR frequencies

Reproductive status	Frequency of TVOR	Fluorescent staining technique					
		Hoechst 33342			FDA		
		No of oocytes kept for staining	No. of matured oocytes	Nuclear maturation rate (%)	No of Oocytes kept for staining	No. of matured oocytes	Oocyte viability rate (%)
Normal breeders	Once weekly	8	6	75	8	7	87.5
	Twice weekly	12	10	83.33	10	8	80
	Total	20	16	80	18	15	83.33
Repeat breeders	Once weekly	9	7	77.78	9	7	77.78
	Twice weekly	12	9	75	11	8	72.73
	Total	21	16	76.19	20	15	75

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