Haryana Vet. (December, 2022) **61(2),** 223-225

Research Article

EFFECT OF DIFFERENT *IN VITRO* MATURATION MEDIUM ON CLEAVAGE AND BLASTOCYST FORMATION RATE IN OVUM PICK-UP DERIVED OOCYTES OF SAHIWAL COWS

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Received: 21.02.2022; Accepted: 06.04.2022

ABSTRACT

Total 51 ovum pick-up sessions were conducted in six Sahiwal donor cows from December, 2020 to December, 2021, and aspirated total 388 oocytes. Of them total 112 (86.15 %), 110 (85.94%) and 121 (93.08%) viable oocytes were subjected to *in vitro* maturation in Group – I (TCM-199), Group – II (Ham's F10) and Group – III (Commercial Medium), respectively. The cleavage rate of 89 (79.46%), 82 (74.55%) and 112 (92.56%) was observed for the oocytes *in vitro* matured in Group - I, Group - II and Group - III, respectively and showed statistically significant difference (P = 0.002). The overall blastocyst production rate was noted to be 35 (33.66%), 29 (27.36%) and 41 (34.17%), in Group - I, Group - II and Group - III, respectively.

Keywords: *In vitro* embryo production, Ovum pick-up, Sahiwal cows

How to cite: Kose, M.S., Patil, M.S., Sahatpure, S.K., Raghuwanshi, D.S., Gawande, A.P., Kurkure, N.V. and Korde, J.P. (2022). Effect of different *in vitro* maturation medium on cleavage and blastocyst formation rate in ovum pick-up derived oocytes of Sahiwal cows. *Haryana Vet.* **61(2)**: 223-225.

Globally the milk production demand is projected to increase by 22% in 2027 compared to 2015-2017 (FAO, 2018). The average yield per animal per day for exotic/crossbred is 7.95 kg per day and for indigenous/non descript it is 3.01 kg per day. In Maharashtra the average yield per animal per day for exotic/crossbred is 9.6 kg per day and for indigenous/non descript it is 2.3 kg per day. To propagate the best available germplasm of high milk producing animals with special reference to indigenous cows, available biotechnological tools include artificial insemination, *in vivo* embryo production and transfer and *in vitro* embryo production and transfer.

With transvaginal oocyte aspiration from live high pedigree donor cows and subsequent *in vitro* maturation of these aspirated oocytes in defined maturation, fertilization and culture medium, *in vitro* embryo production (IVEP) can be achieved. These *in vitro* produced embryos can be transferred to the recipient cows to achieve successful pregnancy. However, the high cost of commercially available *in vitro* maturation, fertilization and culture medium restrict its use at field level. Therefore, to minimize the cost of IVEP, it is proposed to evaluate the effect of different *in vitro* maturation medium on subsequent IVEP in Sahiwal cows.

MATERIALS AND METHODS

The research work was carried out at Department of Animal Reproduction, Gynaecology and Obstetrics, Nagpur Veterinary College, Nagpur from December, 2020 to December, 2021. A total of six Sahiwal cows having lactational yield above 3500 litters, BCS of more than 3 (scale 1-5) and free from any infectious disease condition were selected as Donor cows.

The donor cows (n=6) received an intravaginal progesterone device (P4: 1.38 gm; Eazi Breed CIDR® intravaginal implant; Boehringer Ingelheim, India) along with Estradiol Benzoate (EB) (2 mg, im) (Pregheat, Virbac Animal Health, India) at the random stage of the estrous cycle (day 0). From day 4 onwards, donor cows were stimulated by intra muscular administration of total 200 mg FSH (Folltropin®-V, Vetoquinol N.A. Inc, Canada), divided into four tapering doses (57 mg, 57 mg, 43 mg, 43 mg, respectively, im) given at 12 hours intervals. On Day 8 (52 hr of "coasting" period), ovum pick-up (OPU) was performed along with removal of P4 device (CIDR). Total 51 OPU sessions were performed at an interval of 30 days.

Ovum pick-up (OPU) was performed with the help of transvaginal ultrasound probe having oocyte aspiration assembly attached to it with aspiration pump (WTA, Brazil). Following epidural anaesthesia by using Inj. Lignocaine Hydrochloride (2%), the donor cows were restrained in a service crate. By aspirating the visible ovarian follicles above 5mm on ultrasound monitor (Exago, IMV, France), the content was retrieved in 50 ml conical tube attached to the aspiration pump with the help of 18 G disposable needle in OPU media (IVF Bioscience, UK). The content was immediately transferred into the laboratory. Under stereo zoom microscope, the retrieved content was screened for the presence of oocytes following filtration through 0.75µm steel mesh (Em'con Embryo Filter). The collected

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oocytes were graded in to Grade-A to Grade-D depending on the presence of cumulus cell layers surrounding it.

Briefly, the collected oocytes from Grade-A to Grade-C were submitted to one of the three *in vitro* maturation medium *viz*. Group-I (TCM-199) supplemented with 10% Fetal Calf Serum+5 µg/ml FSH (Folltropin®-V, Vetoquinol N.A. Inc, Canada); Group-II (Ham's F 10) supplemented with 10% Fetal Calf Serum + 5 µg/ml FSH (Folltropin®-V, Vetoquinol N.A. Inc, Canada) and Group-III (Commercial Media) (IVF Bioscience, UK). Following wash in respective maturation medium, the oocytes were transferred to 100 µl droplet of one of the three maturation medium covered under mineral oil and placed for *in vitro* maturation for next 22-24 hours in a benchtop CO_2 incubator having 5% CO_2, 5% O_2 and 90% Nitrogen gas supply at 38.5ºC.

Following incubation for 22-24 hours, the matured oocytes were washed in wash medium and transferred to commercially available *in vitro* fertilization medium (IVF Bioscience, UK). The frozen semen straw of pedigree bull was thawed and the semen was layered on Upper and Lower isolate (FUJIFILM Irvine Scientific) medium, centrifuged at 5000 RPM for 5 minutes and the highly motile spermatozoa were isolated. The 10 µl semen having concentration of 5×10^6 spermatozoa were inoculated in the *in vitro* fertilization droplet of 90µl and incubated for next 18 hours in a benchtop CO_2 incubator having 5% CO_2, 5% O_2 and 90% Nitrogen gas supply at 38.5ºC.

Following 18 hours of incubation in *in vitro* fertilization medium, the presumptive zygotes were washed in wash medium and repeatedly striped off from a 135 µm diameter glass pipette attached to a pipette handle so as to strip out the attached COCs and were placed immediately in commercially available *in vitro* culture medium (IVF Bioscience, UK) for next 8 days. On 2nd day of *in vitro* culture, the presumptive zygotes were observed under stereo zoom microscope to access the cleavage rate. After 8th day of *in vitro* culture the expanded blastocyst production rate was accessed.

Statistical Analysis: The collected data was analysed by using Chi-Squre Test as per Snedecor and Cochran. Means were considered to be statistically different when $P < 0.05$.

RESULT AND DISCUSSION

As shown in Table No. 1, total 51 ovum pick-up sessions were conducted in 6 six Sahiwal donor cows aspirating total 388 oocytes.

In the present study, a total of 112 (86.15 %), 110 (85.94%) and 121 (93.08%) viable oocytes were subjected to *in vitro* maturation in Group-I, Group-II and Group-III, respectively. The cleavage rate of 89 (79.46%), 82 (74.55%) and 112 (92.56%) was observed for the oocytes *in vitro* matured in Group-I, Group-II and Group-III, respectively and showed statistically significant difference (P=0.002). The overall blastocyst production rate was noted to be 35 (33.66%), 29 (27.36%) and 41 (34.17%), in Group-I, Group-II and Group-III, respectively.

Table 1. The ovum details of downer cows

Parameters	Different Culture Media Used			P-Value
	Group-I (TCM-199)	Group-II (Ham's F 10)	Group-III (Commercial Media)	
Number of OPUs	19	17	15	
Total Oocytes retrieved	130	128	130	-
Grade - A	60 (46.15)	63 (49.22)	71 (54.62)	-
Grade - B	41(34.54)	35(27.34)	36(27.69)	-
Grade - C	10 (7.69)	12 (9.38)	15 (11.54)	-
Grade - D	19(14.62)	18(14.06)	8(6.15)	-
Total no. of viable oocytes (%)	112 (86.15)	110 (85.94)	121 (93.08)	0.12
Cleavage rate (%)	89 (79.46)[a]	82 (74.55)[b]	112 (92.56)[c]	0.002*
Blastocyst formation rate (%)	35 (33.66)	29 (27.36)	41 (34.17)	0.49

Our findings are in accordance with the earlier findings of Tutt *et al.* (2021) who stated that ovarian stimulation leads to a significant increase in the yield of high-quality oocytes and potentially transferrable blastocysts. Viable oocytes percentage as observed in the present study are in agreement with the earlier findings of Pontes *et al.* (2009) who recorded 89.30% viable oocytes in Nellore cows and 80.40% as recorded by Tribulo *et al.* (2017). Cavalieri *et al.* (2017) recorded 62.00-65.00% viable oocytes in *Bos indicus* cows which is comparatively lower than the findings of present study. The cleavage rate as observed in the present study are in agreement with the earlier findings of Alvarez *et al.* (2021) who observed the cleavage rate of 85.9, 83.7 and 82.9%, respectively in young, long-lived and senescent cows, observations of Gianluca *et al.* (2003) who recorded cleavage rate of 83.90%, Choi *et al.* (2013) who obtained the cleavage rate of 77-83% in bovine and Zolini *et al.* (2019) who recorded cleavage rate of 83.00 to 84.00% in Holstein virgin heifers.

Comparatively lower (69.00 %) cleavage rate was noted by Hashimoto (2009), Riaz *et al.* (2021) who recorded cleavage rate of 68.60% in sahiwal cows, 33.00% as recorded by Manik *et al.* (2003), 59.40% as recorded by Tribulo *et al.* (2017) and Hendriksen *et al.* (2004) who recorded cleavage rate of 45.00 to 75.00% in cows.

Our findings of blastocyst production rate are in accordance with the earlier findings of Hashimoto (2009) who also recorded blastocysts rate of 37.00%, Riaz *et al.* (2021) who recorded blastocyst rate of 26.30%, 22-39% blastocyst formation rate as recorded by Goodhand *et al.*

(1999), De Roover *et al.* (2005) who also recorded blastocyst formation rate of 24-29%, blastocyst formation rate of 32.00% as recorded by Tribulo *et al.* (2017), Pontes *et al.* (2009) who recorded 41.10% blastocysts in Nellore cows and Lopes *et al.* (2006) who recorded 25.00% blastocyst formation rate.

Comparatively higher blastocyst formation rate (64.8-65.00 %) was recorded by Alvarez *et al.* (2021) in young and long-lived cows and Gianluca *et al.* (2003) as 49.20% in bovine. Blastocyst formation rate of 19.00% as reported by Zolini *et al.* (2019) in Holstein virgin heifers is comparatively lower than the observations in the present study.

CONCLUSION

Stimulation of the donor cows with FSH resulted in more number of viable oocytes. Significantly higher cleavage rate was observed for the oocytes matured in Commercial medium than in the TCM – 199 and Ham's F10 medium. Blastocyst formation rate was non-significantly different for the oocytes matured in different maturation medium.

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