Supplementation of Buffalo Follicular Fluid: Beware of Other Sources of Steroid Hormones during Culture

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ABSTRACT

An attempt was made to describe the updated information about the factors to be concerned before using of buffalo follicular fluid for supplementation \textit{in vitro} of buffalo oocyte. There are not only diameter of the follicle, the present of corpus luteum (CL), growth of follicles, stage of estrus cycle, healthy and atretic state of the ovarian follicles. The progesterone and estradiol-17B concentration of follicular fluid from individual laboratory have to be determined for optimization of follicular fluid for supplementation. Fortunately, the effect is not severe because of the mixture of fluid from various stages of oestrous cycle. It is suggested to pre-determine the progesterone and estradiol-17\(\beta\) concentration of follicular fluid from different size of follicles and the present of CL, whose ovaries, retrieved from slaughterhouse, were at unknown estrus cycle. The other natural sources of progesterone and estradiol-17\(\beta\) were also discussed in this study. Therefore, the amount of supplementation may be not as high as expected. In conclusion, optimization of follicular fluid is suggested. This might be lead to reduce the amount of chemical steroids supplementation. Hence, the cost of expense is reduced.

Keywords: steroid, follicular fluid, follicle, corpus luteum, buffalo, progesterone, estradiol-17\(\beta\)

INTRODUCTION

Supplementation of natural steroid was demonstrated in culture medium with biological compounds has been demonstrated to be advantages for embryo development in vitro (Carolan et al., 1995). Results, however, have been varied in optimal concentration of buffalo follicular fluid used. Furthermore, other sources of natural steroid, apart from follicular fluid, were described. Therefore, the amount of supplementation may be not as high as expected. The objective of this study was to suggest some points to be concerned, in order to optimize the amount of follicular fluid.

Various Amount Of Follicular Fluid Use For Supplementation

It is noted that the optimal amount used of follicular fluid for \textit{in vitro} maturation are varied (Table I) from 10\% (Yadav et al., 1997; Tajik et al., 2000 Abdoon,2002), 20\% (Das et al., 1996; Chauhan et al., 1997; Tajik et al.,2000) till 100\% (Gupta et al., 2001; Nandi et al., 2004). Furthermore, the reports on follicle size are also varied (Table I). Nandi et al. (2004) showed the better results when they used follicular fluid from small follicle (< 3 mm in diameter), whereas Gupta et al. (2001) preferred follicle > 5 mm in diameter, Yadav et al. (1997) chose 2-12 mm in diameter for their experiments, that was similar to Chauhan et al. (1997) who used 4-10 mm. in diameter. One of the effects on such variability might be the level of hormonal concentration in the follicular fluid at the time of aspiration. Our results showed that there were strongly relationship between follicle size and the presence of corpus luteum(CL). This study suggested that follicular Accepted April 10, 2013; Online February 24, 2014.
fluid from the ovaries with CL of the follicle both size should not be suitable for IVM. Because the high level of progesterone inhibits estrus and the ovulatory surge of LH (Hafez and Hafez, 2000). The concentration of the progesterone in the follicular fluid is critical for oocyte maturation (Atheya and Totey, 2002).

Our previous results (Srisakwattana et al., 2005) also suggested that the follicular fluid from large size of follicle of the ovaries without CL is tending to preferable to use in oocyte maturation than those from small follicles because our data showed that the content of estradiol-17β of the large follicles was much higher than those of the small follicles at the time of aspirating. The progesterone of the follicles with CL was higher than those from follicles without CL (Srisakwattana et al, 2005).

Kulkarni et al. (1994) also reported the wide range of progesterone and estradiol-17β concentration in their three sizes of follicles.

Our previously study (Srisakwattana et al., 2005) showed that as the follicular size of the ovaries without CL increased, the progesterone and estradiol-17β levels in the follicular fluid increased. This results was also corresponded to other’s results (Eissa et al, 1995, 1996; Hooda and Yadav, 2002; Atheya and Totey, 2002) that as the follicular size increased, follicular fluid concentration of progesterone and estradiol-17β significantly increase. Hooda and Yadav (2002) also reported that the increases in estradiol-17β concentration with the increase in follicle size in their study could be because of the cumulative effect of increasing aromatase activity per cell and increasing cell number.

These indicated that stage of follicle development and their size and the presence of CL were possibly among the factors that resulted in such a wide range amount of the % follicular fluid used for in vitro oocyte maturation have been reported (Table 1).

**Other Sources of Natural Steroids**

Although, the concentrations of estradiol and progesterone in fetal bovine serum (FBS) are low, however, their presence should be considered when FBS is included in culture systems (Stubbing et al., 1989; Gordon 2003; van de Valk et al., 2010). Cumulus cells of bovine COCs matured in vitro are able to secrete estradiol and progesterone when matured in medium supplemented with bovine serum albumin (BSA) and gonadotrophins, and that this steroidogenesis can be modulated by steroids (Mingoti et al., 2002) and they also reported that BSA was also contaminated with progesterone.

It was reported that granulose cell of follicle with the presence of CL, when cultured with FBS, showed a higher peak of secreted P4 (22.65, 29.96 ng/ml) than those of without CL (17.12, 18.6 ng/ml) (Srisakwattana et al., 2006). Acyclic buffaloes have lower concentrations of estradiol and insulin concurrent with higher concentrations of progesterone in the follicular fluid (Khan et al., 2012). In ovaries, the maturation of oocyte is also governed by estrogen produced by the granulosa cells (Atheya and Totey, 2002).

**Table 1.** Various concentrations of buffalo follicular fluid and follicle diameter used for in vitro maturation, fertilization and subsequent embryo development.

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<tr>
<td>Follicular fluid used (%)</td>
<td>5%</td>
<td>10%</td>
<td>100%</td>
<td>10% 50% 100%</td>
<td>10% 20%</td>
<td>20% 40%</td>
<td>10%</td>
<td>10%</td>
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<tr>
<td>Follicle diameter (mm)</td>
<td>2-8</td>
<td>&lt;3 3-8 &gt;8</td>
<td>-</td>
<td>&gt;3</td>
<td>-</td>
<td>4-10</td>
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CONCLUSION

Optimization of follicular fluid, whose the ovaries were at unknown estrous cycle, is suggested. Therefore, follicular fluid should be classified according to the size of follicles whose oocytes are retrieved. This should help to select the more appropriate source of follicular fluid for supplementation in order to obtain higher maturation rate and subsequent embryo development. Apart from other factors included in follicular fluid should be aware. It is suggested that each batch of follicular fluid should have preliminary information on progesterone and estradiol-17B concentrations. The amount of supplementation may be not as high as expected. Therefore, the addition of commercial steroid for supplementation might be reduced. Hence, the cost of chemical expense will be decreased.

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