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SAFETY OF DIFLUBENZURON TREATMENT IN BOVINE OOCYTE DONORS

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BACKGROUND-AIM

There is an increasing concern about the potential negative effects on fertility of chemicals used in agriculture and livestock. The diflubenzuron (1-(4-chlorophenyl)-3-(2,6-difluoro-benzoyl) urea, DFB) is an insect growth regulator used in the control of ectoparasites in both dairy and beef herds. The toxicity of DFB, however, remains controversial. In this regard, in vitro embryo production (IVEP) offers an excellent experimental model for toxicity studies, both considering the sensitivity of gamete and embryo culture systems, and the scale used. The aim of this study was to evaluate the safety of DFB in bovine oocyte donors and subsequent impact on IVEP.

METHODS

Sound, nulliparous Nelore (*Bos taurus indicus*) heifers (n=16) were allocated into a control (CG) or treatment (DFB) groups. The heifers were confined and received 100g/head/day of a mineral mix supplement containing (DFB) or not (CG) 1.2 g diflubenzuron 3% (Difly, Champion, Brazil). Heifers were weighed and blood samples were collected weekly, during nine weeks, for analysis of hematological and biochemical endpoints. Heifers were subject to five ovum pick-up sessions performed every other week, beginning just before DFB treatment (week 0). The COC recovered were morphologically evaluated and those classified as viable were sent to an IVEP laboratory. Data were analyzed considering the effects of treatment, time, and their interaction, using the Proc Glimmix of the SAS.

RESULTS

There was no effect of DFB treatment on average body weight (P=0.9396), hematocrit (P=0.1632), or in plasma protein (P=0.6144), alkaline phosphatase (P=0.7731), creatinine (P=0.7605) or urea (P=0.1199). In both groups, all blood endpoints remained within the physiological range throughout the experimental period. There were no differences between CG and DFB groups in the number of total (P=0.3694), viable (P=0.3947) or grade I oocytes (P=0.3242), as well as in cleavage (P=0.2893) or blastocyst rates (P=0.5301). Similarly, the proportion of expanded blastocysts (P=0.7913) or the number of embryos with more than 100 cells (P=0.9452) were similar between groups.

CONCLUSIONS

The oral administration of diflubenzuron, within the recommended dose, has no short-term negative effects on oocyte quality or in vitro developmental competence in cattle.

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USE OF HUMAN RECOMBINANT FSH FOR IN VITRO EMBRYO PRODUCTION IN CATTLE

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BACKGROUND-AIM

The recombinant human FSH (rhFSH) has been used during in vitro maturation (IVM) of cattle oocytes as an alternative to replace FSH obtained from porcine pituitary (pFSH) and available for veterinary use. Among the advantages of the rhFSH are the lower variation in biological activity and the lack of sanitary risks associated with the use of protein extracts from other species. However, few studies have directly compared the efficiency of rhFSH and pFSH for IVM in cattle. In this study we evaluated in vitro embryo production outcomes using both sources of FSH.

METHODS

Bovine cumulus-oocyte complexes (COC, n=945) recovered from slaughterhouse ovaries and morphologically classified as grades I or II were used. The COC were allocated into three groups, which were IVM in TCM 199 medium: (1) without FSH (-FSH n=315), (2) with 0.5 µg/mL pFSH (Folltropin-V, Vetoquinol, n=315), or (3) with 0.1 UI/mL rhFSH (Gonal, Merck, n=315), under the same culture conditions (38.5°C, 5% CO₂). Cumulus expansion was addressed after 22 h of IVM and subjectively graded as poor, average, or good. Semen from a sire with known fertility was used for in vitro fertilization. The presumptive zygotes were cultured under low oxygen tension (5% CO₂ and 5% O₂, 38,5°C). Cleavage, blastocyst, and hatching rates were evaluated at days 3, 7, and 10 of culture, respectively. Embryo production data were analyzed using the Proc GLIMMIX of the SAS (SAS Institute), and cumulus expansion using the Kruskal-Wallis test.

RESULTS

As expected, cumulus expansion grade was greater (P<0.0001) when FSH was used, regardless the source. There was a significant effect of treatment, but not of replica or treatment*replica, on cleavage and blastocyst rates. The cleavage (day 3) rate was similar (P=0.7339) between groups -FSH and pFSH, but in both groups rates were lower (P<0.05) when compared with rhFSH (71.5% and 70.0% vs. 80.4; respectively). The blastocyst rate was affected (P<0.05) by the source of FSH (34.6%a, 38.0%ab and 46.0%b for -FSH, pFSH and rhFSH, respectively). However, there was no difference (P>0.05) in hatching rates among groups, which only varied (P=0.0024) according to replica.

CONCLUSIONS

The rhFSH improve blastocyst rates and therefore is an alternative to pFSH for IVM of cattle COC.

ABSTRACT BOOK



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